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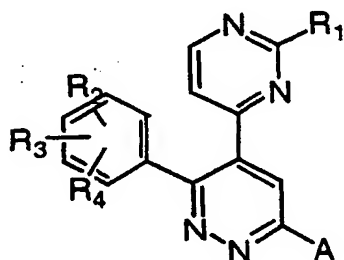
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(54) Title: SUBSTITUTED PYRIDAZINES HAVING CYTOKINE INHIBITORY ACTIVITY



(I)

(57) Abstract: There are disclosed compounds of formula (I) and pharmaceutically acceptable salts thereof which exhibit utility for the treatment of cytokine mediated diseases such as arthritis.

WO 01/42241 A1

TITLE OF THE INVENTION

SUBSTITUTED PYRIDAZINES HAVING CYTOKINE INHIBITORY ACTIVITY

BACKGROUND OF THE INVENTION

5 The present invention relates to substituted pyridazine compounds which have cytokine inhibitory activity. Cytokine mediated diseases and cytokine inhibition, suppression and antagonism are used in the context of diseases or conditions in which excessive or unregulated production or activity of one or more cytokines occurs. Examples of cytokines which are effected typically include
10 Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor (TNF).

 Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) are produced by a variety of cells that are involved in immunoregulation and other physiological conditions.

15 There are many disease states in which IL-1 is implicated. Examples are rheumatoid arthritis, osteoarthritis, endotoxemia, toxic shock syndrome, acute and chronic inflammatory diseases, such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease; tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, rheumatoid arthritis,
20 gout, traumatic arthritis, rubella arthritis and acute synovitis. Recent evidence also links IL-1 activity to diabetes.

 Interleukin-1 has been demonstrated to mediate a variety of biological activities thought to be important in immunoregulation and other physiological conditions. [See, e.g., Dinarello et al., Rev. Infect. Disease, 6, 51
25 (1984)]. The known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, neutrophil chemotaxis, induction of acute phase proteins and the suppression of plasma iron levels.

 Excessive or unregulated tumor necrosis factor (TNF) production
30 or activity has been implicated in mediating or exacerbating rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion
35 injury, graft v. host rejection, allograft rejections, fever and myalgia

due to infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis.

Monokines, such as TNF, have also been shown to activate HIV replication in monocytes and/or macrophages [See Poli, *et al.*, Proc. Natl. Acad. Sci., 87:782-784 (1990)], therefore, inhibition of monokine production or activity aids in limiting HIV progression. TNF has been implicated in various roles with other viral infections, such as the cytomegalovirus (CMV), influenza virus and the herpes virus.

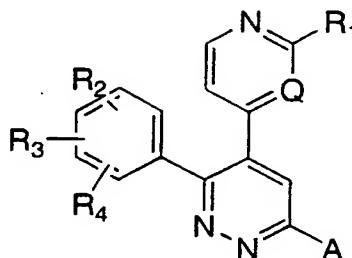
Interleukin-6 (IL-6) is a cytokine effecting the immune system and hematopoiesis. It is produced by several mammalian cell types in response to agents such as IL-1, and is correlated with disease states such as angiofollicular lymphoid hyperplasia.

Interleukin-8 (IL-8) is a chemotactic factor first identified and characterized in 1987. Many different names have been applied to IL-8, such as neutrophil attractant/activation protein-1 (NAP-1), monocyte derived neutrophil chemotactic factor (MDNCF), neutrophil activating factor (NAF), and T-cell lymphocyte chemotactic factor. Like IL-1, IL-8 is produced by several cell types, including mononuclear cells, fibroblasts, endothelial cells and ketainocytes. Its production is induced by IL-1, TNF and by lipopolysaccharide (LPS). IL-8 stimulates a number of cellular functions *in vitro*. It is a chemoattractant for neutrophils, T-lymphocytes and basophils. It induces histamine release from basophils. It causes lysozomal enzyme release and respiratory burst from neutrophils, and it has been shown to increase the surface expression of Mac-1 (CD11b/CD 18) on neutrophils without *de novo* protein synthesis.

There remains a need for compounds which are useful in treating cytokine mediated diseases, and as such, inhibit, suppress or antagonize the production or activity of cytokines such as IL-1, IL-6, IL-8 and TNF.

SUMMARY OF THE INVENTION

The present invention relates to compound I of the formula



(I)

wherein

5

A is halogen, phenyl, S(O)_m phenyl, or NR₅R₆;

R₁ is hydrogen, NH(C₁-C₆ alkyl)aryl, NH(C₁-C₆ alkyl) or NH(C₃-C₆ cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF₃, NH₂, and NO₂;

10

R₂, R₃ and R₄ independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF₃, NH₂, NO₂, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₈ cycloalkyl or phenyl;

15

R₅ and R₆ are independently hydrogen, C₁-C₆ alkyl, (C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), (C₁-C₆ alkyl)cycloalkyl, (C₁-C₆ alkyl) NR₇R₈, C₁-C₆ alkylphenyl, said phenyl group optionally substituted with 1 to 3 groups selected from (C₁-C₆ alkyl) or (C₁-C₆ alkoxy); (C₁-C₆ alkyl)-NHCOO-(C₁-C₆ alkyl), (C₁-C₆ alkyl)C≡C, (C₁-C₆ alkyl)indole, (C₁-C₆ alkyl)pyridinyl, a pyrrolidinyl or piperidyl group, said groups optionally substituted with C₁-C₆ alkyl or benzyl; or

20

R₅ and R₆ are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring containing at least one N atom, and optionally containing 1-2 additional

25

5 N atoms and 0-2 O or S atoms, said ring optionally substituted by 1-3 groups selected from C₁-C₆alkyl, OH, O(C₁-C₆ alkyl), COO(C₁-C₆ alkyl), C₁-C₆alkyl benzodioxole, CONR₇R₈, phenyl, said phenyl group optionally substituted with halogen, C₁-C₆alkyl, C₁-C₆ alkoxy;
 10 CH(aryl)₂ said aryl optionally substituted with 1-3 groups selected from C₁-C₆alkyl, OH or halogen; NR₇R₈ or a piperidino or a pyrrolidino group;

10 R₇ and R₈ are independently hydrogen, C₁-C₆ alkyl, (C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), C₁-C₆ alkylaryl, (C₁-C₆ alkyl)-NHCOO-(C₁-C₆ alkyl), COO-(C₁-C₆ alkyl), a pyrrolidinyl or piperidyl group, said groups optionally substituted with C₁-C₆ alkyl or C₁-C₆ alkylaryl; or

15 R₇ and R₈ are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C₁-C₆alkyl, OH, O(C₁-C₆ alkyl),

20 Q is CH or N;

m is 0, 1 or 2;

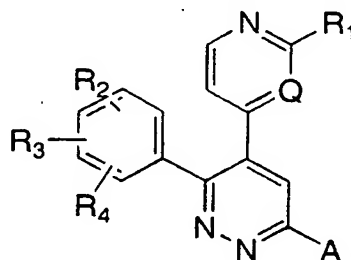
25 or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

This invention also relates to a pharmaceutical composition that is comprised of a compound of formula I as defined above in combination with a pharmaceutically acceptable carrier.

30 Also included in the invention is a method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound of formula I which is effective for treating said cytokine mediated disease.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compound I of the formula



(I)

wherein

A is halogen, phenyl, S(O)_m phenyl, or NR₅R₆;

R₁ is hydrogen, NH(C₁-C₆ alkyl)aryl, NH(C₁-C₆ alkyl) or NH(C₃-C₆ cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF₃, NH₂, and NO₂;

R₂, R₃ and R₄ independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF₃, NH₂, NO₂, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₈ cycloalkyl or phenyl;

R₅ and R₆ are independently hydrogen, C₁-C₆ alkyl, (C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), (C₁-C₆ alkyl)cycloalkyl, (C₁-C₆ alkyl) NR₇R₈, C₁-C₆ alkylphenyl, said phenyl group optionally substituted with 1 to 3 groups selected from (C₁-C₆ alkyl) or (C₁-C₆ alkoxy); (C₁-C₆ alkyl)-NHCOO-(C₁-C₆ alkyl), (C₁-C₆ alkyl)C≡C, (C₁-C₆ alkyl)indole, (C₁-C₆ alkyl) pyridinyl, a pyrrolidinyl or piperidyl group, said groups optionally substituted with C₁-C₆ alkyl or benzyl; or

R₅ and R₆ are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring containing at least one N atom, and optionally containing 1-2 additional

N atoms and 0-2 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1-C_6 alkyl, OH, $O(C_1-C_6 \text{ alkyl})$, $COO(C_1-C_6 \text{ alkyl})$, C_1-C_6 alkyl benzodioxole, $CONR_7R_8$, phenyl, said phenyl group optionally substituted with halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy;

5 $CH(aryl)_2$ said aryl optionally substituted with 1-3 groups selected from C_1-C_6 alkyl, OH or halogen; NR_7R_8 or a piperidino or a pyrrolidino group:

10 R_7 and R_8 are independently hydrogen, C_1-C_6 alkyl, $(C_1-C_6 \text{ alkyl})-O-(C_1-C_6 \text{ alkyl})$, C_1-C_6 alkylaryl, $(C_1-C_6 \text{ alkyl})-NHCOO-(C_1-C_6 \text{ alkyl})$, $COO-(C_1-C_6 \text{ alkyl})$, a pyrrolidinyl or piperidyl group, said groups optionally substituted with C_1-C_6 alkyl or C_1-C_6 alkylaryl; or

15 R_7 and R_8 are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1-C_4 alkyl, OH, $O(C_1-C_6 \text{ alkyl})$,

20 Q is CH or N;

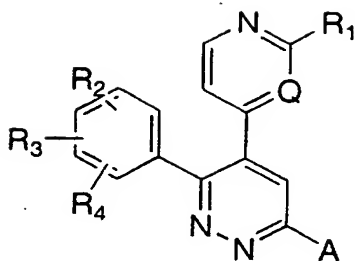
m is 0, 1 or 2;

25 or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

This invention also relates to a pharmaceutical composition that is comprised of a compound of formula I as defined above in combination with a pharmaceutically acceptable carrier.

30 Also included in the invention is a method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound of formula I which is effective for treating said cytokine mediated disease.

In a preferred embodiment, there is disclosed a compound of the formula



(I)

wherein

A is NR_5R_6 ;

5 R_1 is $\text{NH}(\text{C}_1\text{-C}_6 \text{ alkyl})\text{aryl}$, said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3 , NH_2 , and NO_2 ;

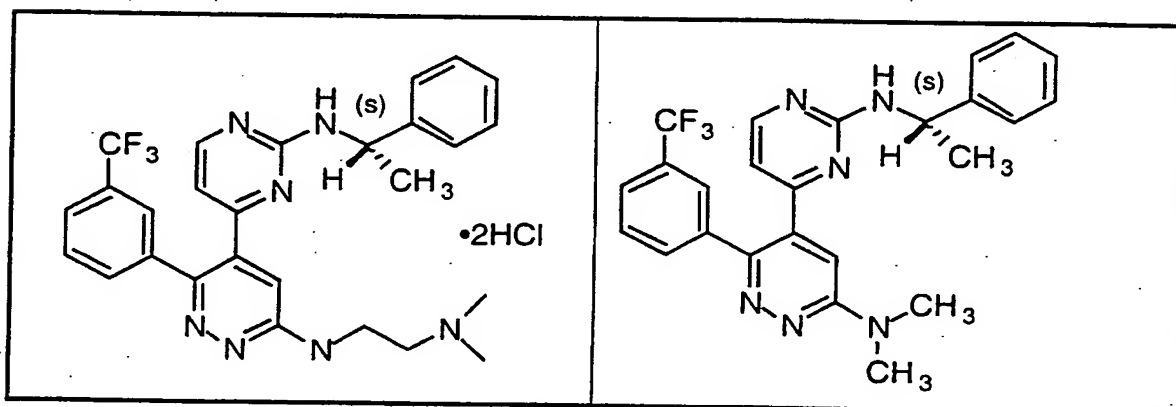
R_2 is CF_3 ;

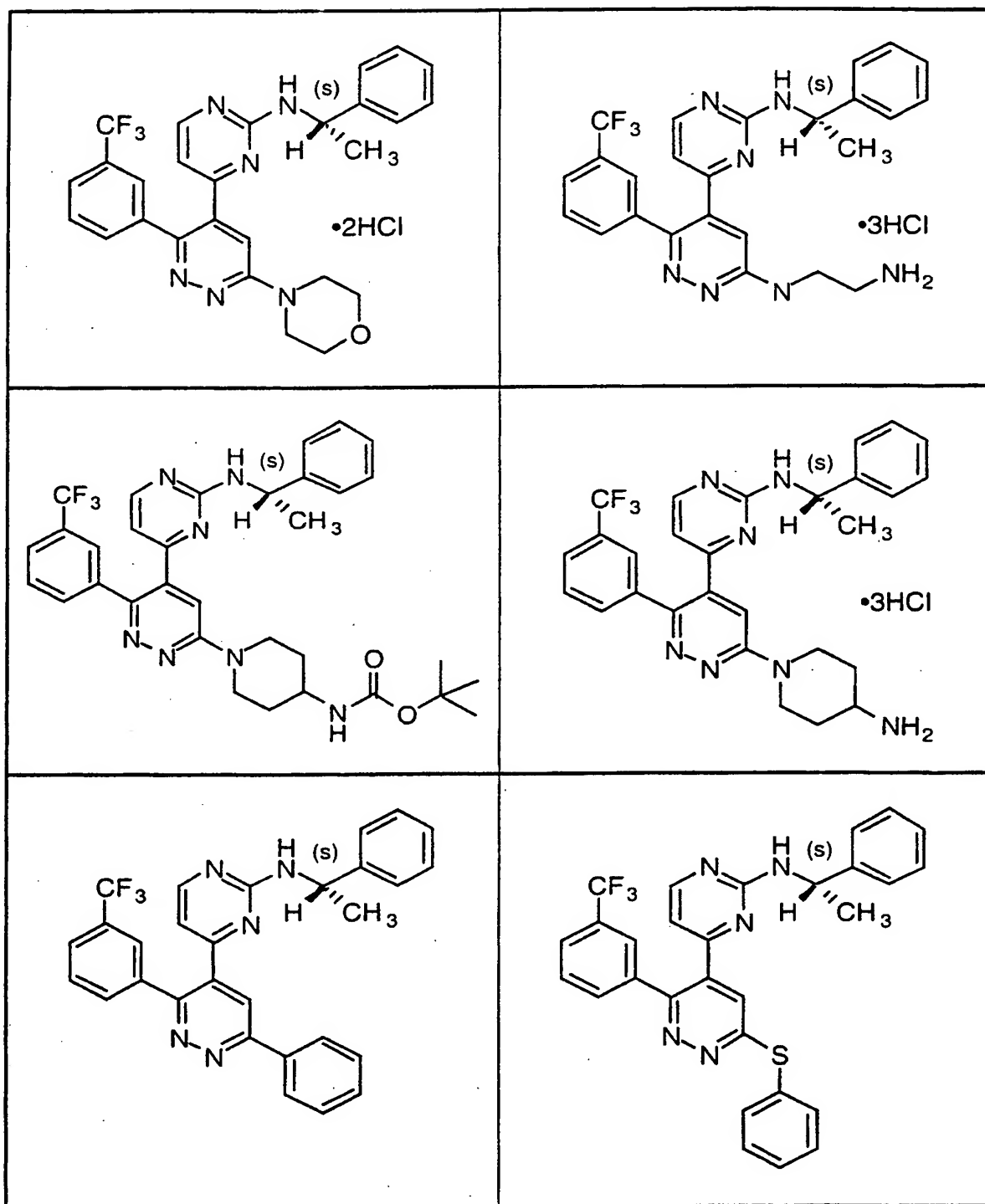
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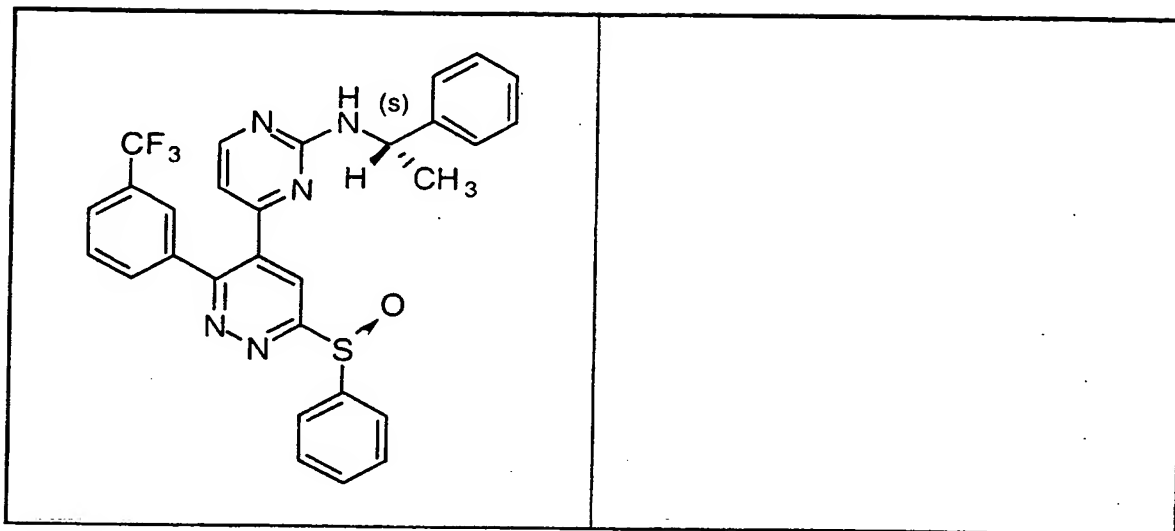
Q is N;

or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

15 Representative species falling within the present invention include the following:



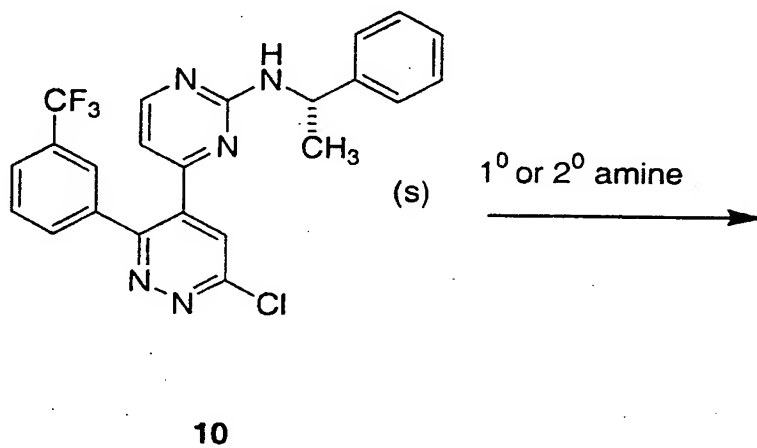


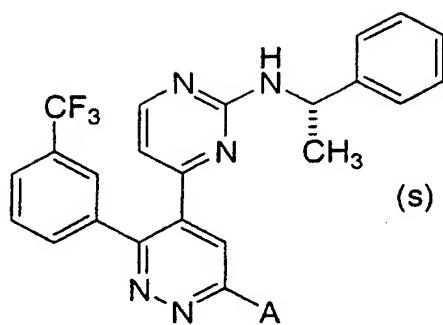


Other representative compounds of the invention include primary and secondary amines wherein A is as shown in the table below:

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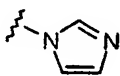
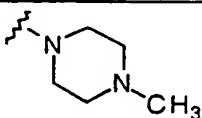
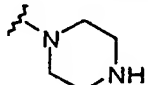
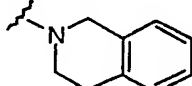
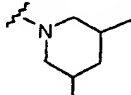
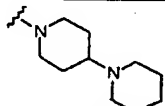
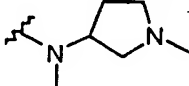
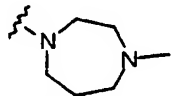
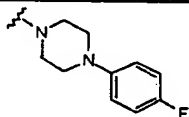
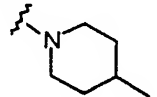
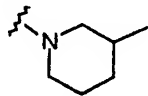
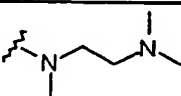
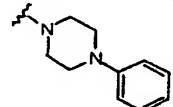
Table 1: Reaction of 10 With 1° or 2° Amines

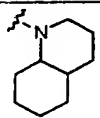
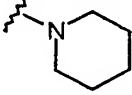
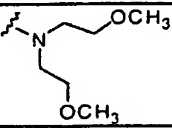
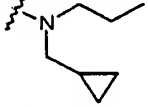
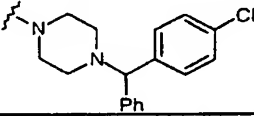
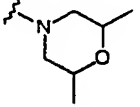
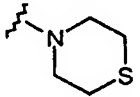
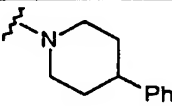
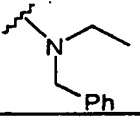
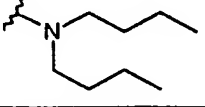
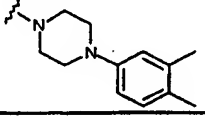
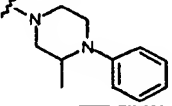
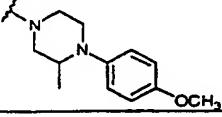


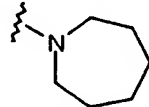
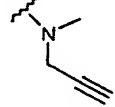
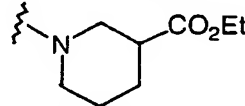
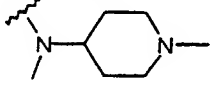
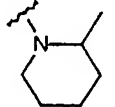
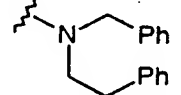
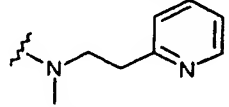
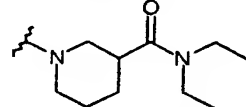
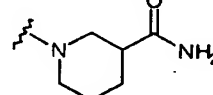
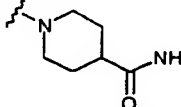
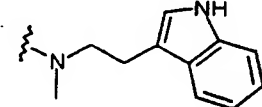
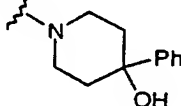
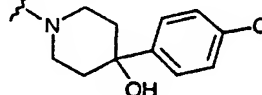


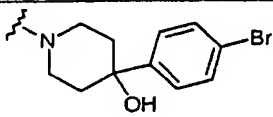
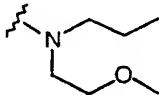
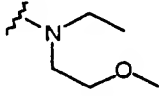
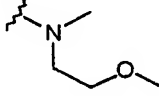
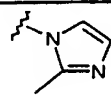
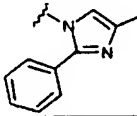
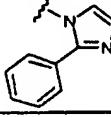
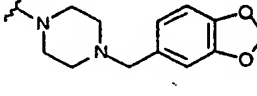
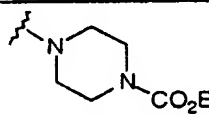
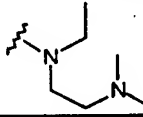
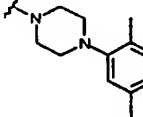
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Unless otherwise stated or indicated, the following definitions shall apply throughout the specification and claims.

The term "alkyl" refers to a monovalent alkane (hydrocarbon) derived radical containing from 1 to 15 carbon atoms unless otherwise defined. It may be straight or branched, and when of sufficient size, e.g., C₃₋₁₅, may be cyclic. Preferred straight or branched alkyl groups include methyl, ethyl, propyl, isopropyl, butyl and t-butyl. Preferred cycloalkyl groups include cyclopropyl, cyclopentyl and cyclohexyl.

Alkyl also includes an alkyl group substituted with a cycloalkyl group, such as cyclopropylmethyl. Alkyl also includes a straight or branched alkyl group.

The alkylene and monovalent alkyl portion(s) of the alkyl group can be attached at any available point of attachment to the cycloalkylene portion.

When substituted alkyl is present, this refers to a straight, branched or cyclic alkyl group as defined above, substituted with 1-3 groups as defined with respect to each variable.

The term "aryl" refers to aromatic rings e.g., phenyl, substituted phenyl and like groups as well as rings which are fused, e.g., naphthyl and the like. Aryl thus contains at least one ring having at least 6 atoms, with up to two such rings being present, containing up to 10 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms. The preferred aryl groups are phenyl and naphthyl. Aryl groups may likewise be substituted as defined below. Preferred substituted aryls include phenyl or naphthyl substituted with one or two groups.

The terms "heterocycloalkyl" and "heterocyclyl" refer to a cycloalkyl group (nonaromatic) in which one of the carbon atoms in the ring is replaced by a heteroatom selected from O, S(O)_m or N, and in which up to three additional carbon atoms may be replaced by said heteroatoms. When three heteroatoms are present in the heterocycle, they are not all linked together.

Examples of heterocyclyls are piperidiny, morpholinyl, azetidiny, pyrrolidinyl, tetrahydrofuranyl, imidazoliny, piperazinyl, pyrrolidin-2-one, piperidin-2-one and the like.

The term "halogen" or "halo" is intended to include fluorine, chlorine, bromine and iodine.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as

any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

In addition, it is well known to those skilled in the art that many of the foregoing heterocyclic groups can exist in more than one tautomeric form. It is intended that all such tautomers be included within the ambit of this invention.

The optical isomeric forms, that is mixtures of enantiomers, e.g., racemates, or diastereomers as well as individual enantiomers or diastereomers of the instant compound are included. These individual enantiomers are commonly designated according to the optical rotation they effect by the symbols (+) and (-), (L) and (D), (l) and (d) or combinations thereof. These isomers may also be designated according to their absolute spatial configuration by (S) and (R), which stands for sinister and rectus, respectively.

The individual optical isomers may be prepared using conventional resolution procedures, e.g., treatment with an appropriate optically active acid, separating the diastereomers and then recovering the desired isomer. In addition, the individual optical isomers may be prepared by asymmetric synthesis.

Additionally, a given chemical formula or name shall encompass pharmaceutically acceptable addition salts thereof and solvates thereof, such as hydrates.

The compounds of the present invention, while effective themselves, may be formulated and administered in the form of their pharmaceutically acceptable addition salts for purposes of stability, convenience of crystallization, increased solubility and other desirable properties.

The compounds of the present invention may be administered in the form of pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" is intended to include all acceptable salts. Examples of acid salts are hydrochloric, nitric, sulfuric, phosphoric, formic, acetic, trifluoroacetic, propionic, maleic, succinic, malonic, methane sulfonic and the like which can be used as a dosage form for modifying the solubility or hydrolysis characteristics or can be used in sustained release or prodrug formulations. Depending on the particular functionality of the compound of the present invention, pharmaceutically acceptable salts of the compounds of this invention include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine,

N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl) amino-methane, and tetramethylammonium hydroxide. These salts may be prepared by standard procedures, e.g. by reacting a free acid with a suitable organic or inorganic base, or alternatively by reacting a free base with a suitable organic or inorganic acid.

5 Also, in the case of an acid (-COOH) or alcohol group being present, pharmaceutically acceptable esters can be employed, e.g. methyl, ethyl, butyl, acetate, maleate, pivaloyloxymethyl, and the like, and those esters known in the art for modifying solubility or hydrolysis characteristics for use as sustained release or prodrug formulations.

10 The compounds of the present invention may have chiral centers other than those centers whose stereochemistry is depicted in formula I, and therefore may occur as racemates, racemic mixtures and as individual enantiomers or diastereomers, with all such isomeric forms being included in the present invention as well as mixtures thereof. Furthermore, some of the crystalline forms
15 for compounds of the present invention may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds of the instant invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of this invention.

20 The term "TNF mediated disease or disease state" refers to disease states in which TNF plays a role, either by production or increased activity levels of TNF itself, or by causing another cytokine to be released, such as but not limited to IL-1 or IL-6. A disease state in which IL-1, for instance is a major component, and whose production or action, is exacerbated or secreted in response to TNF, would
25 therefore be considered a disease state mediated by TNF.

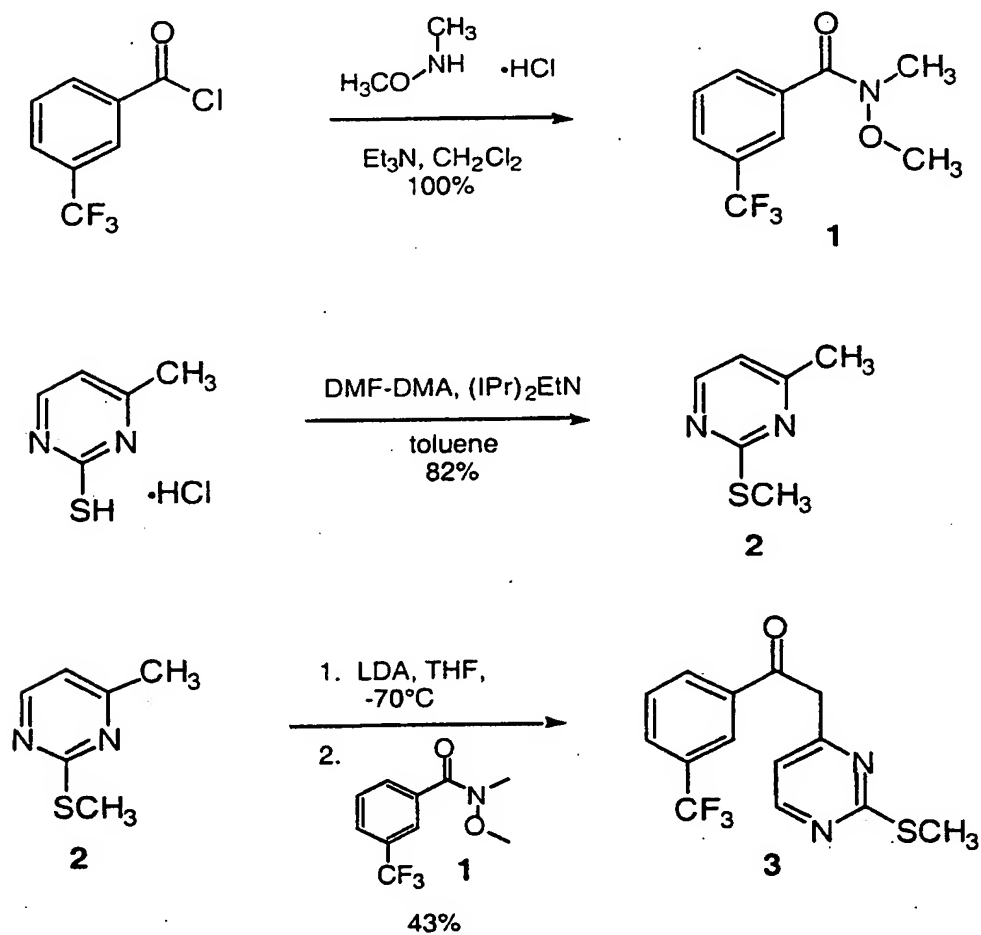
The term "cytokine" as used herein means any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines regardless of which cells
30 produce them. Examples of cytokines include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF- α) and Tumor Necrosis Factor-beta (TNF- β).

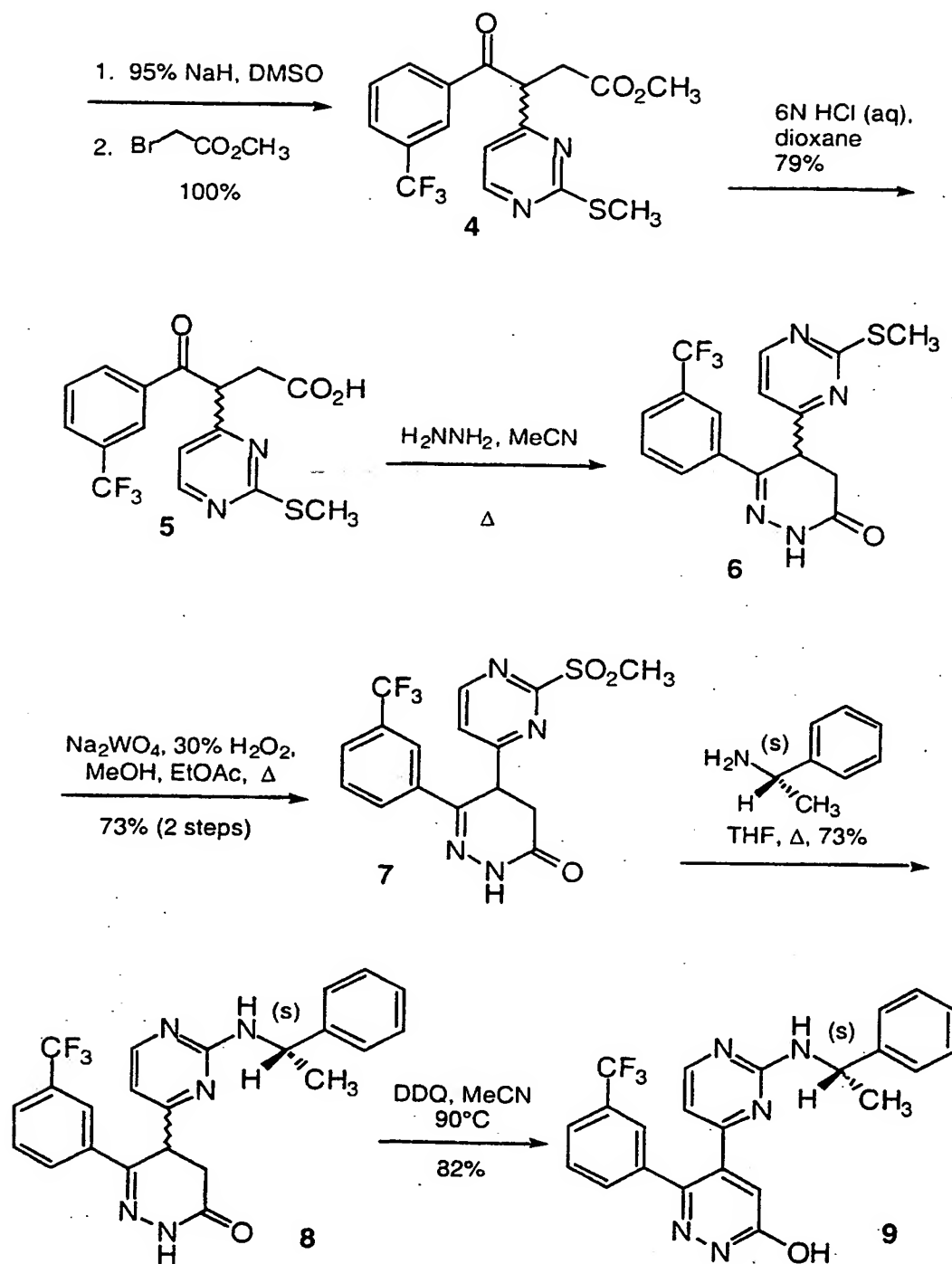
By the term "cytokine interfering or cytokine suppressive amount" is meant an effective amount of a compound of formula I which will cause a decrease

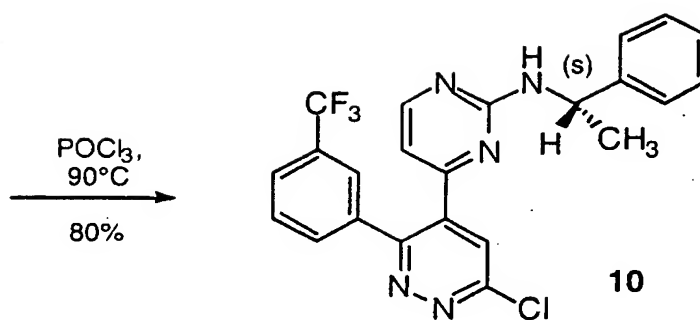
in the *in vivo* activity or level of the cytokine to normal or sub-normal levels, when given to the patient for the prophylaxis or therapeutic treatment of a disease state which is exacerbated by, or caused by, excessive or unregulated cytokine production or activity.

- 5 The compounds of the invention are prepared by the following reaction scheme(s). All substituents are as defined above unless indicated otherwise.

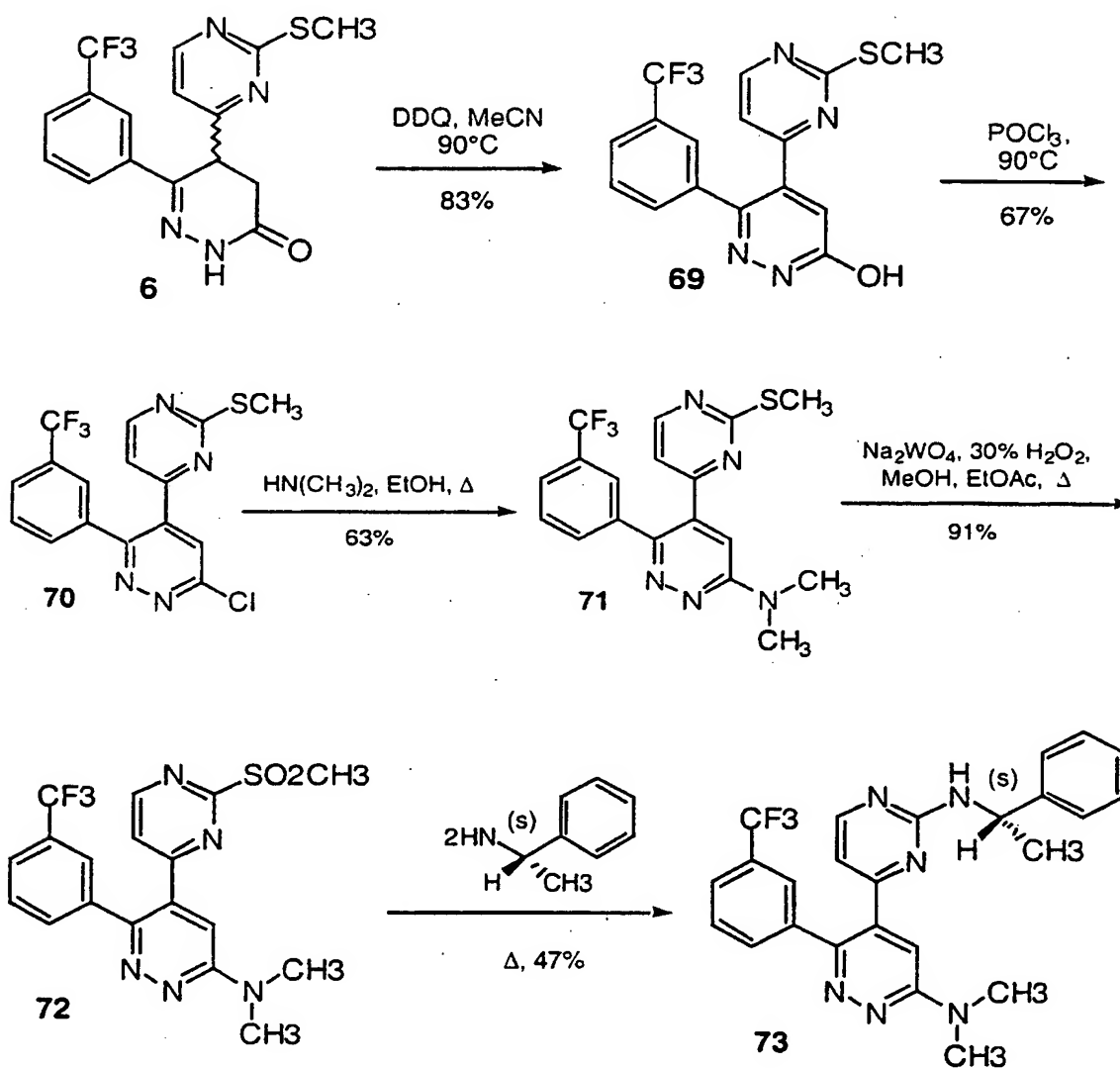
Scheme 1: Synthesis of Compound 10



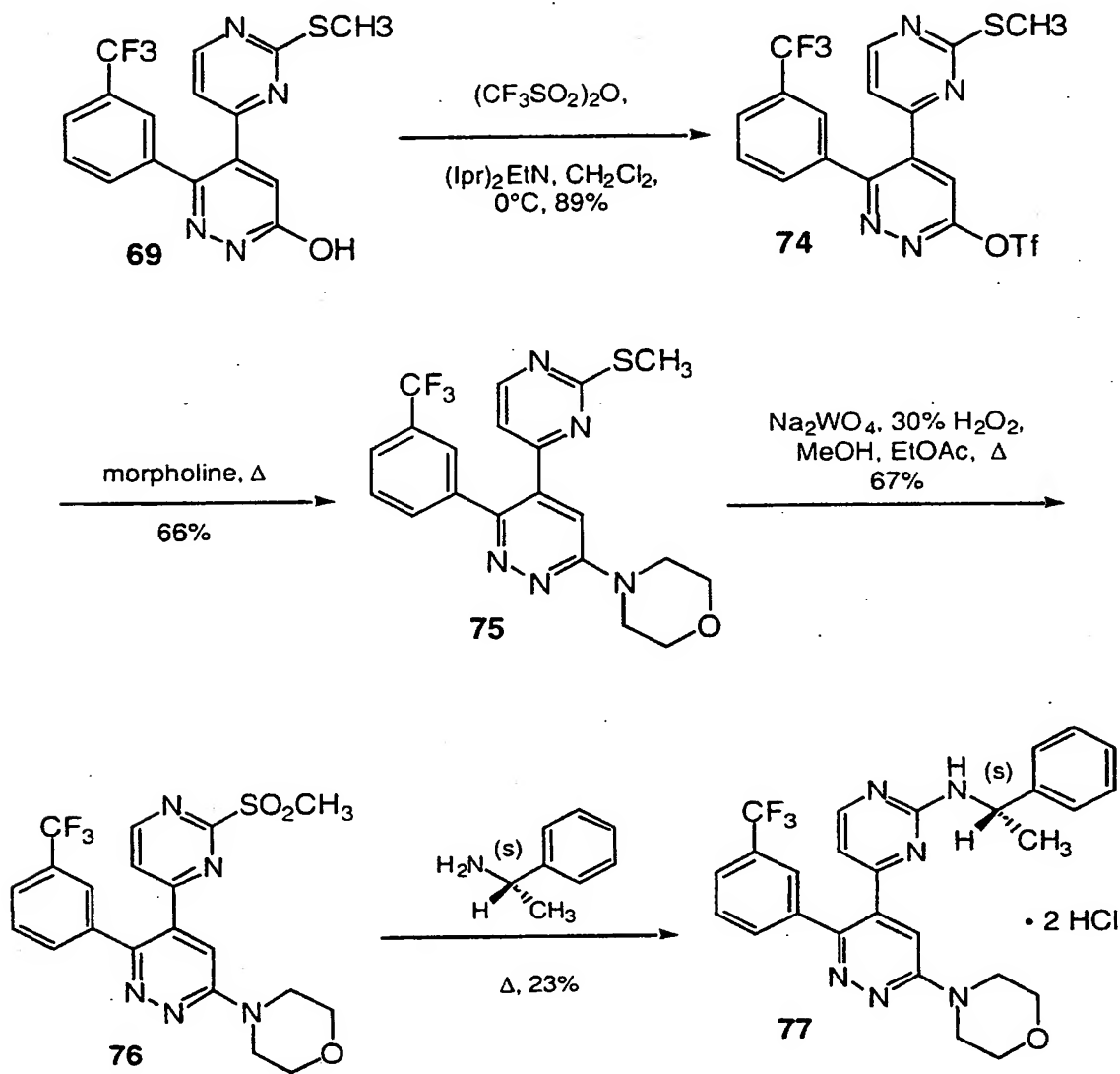




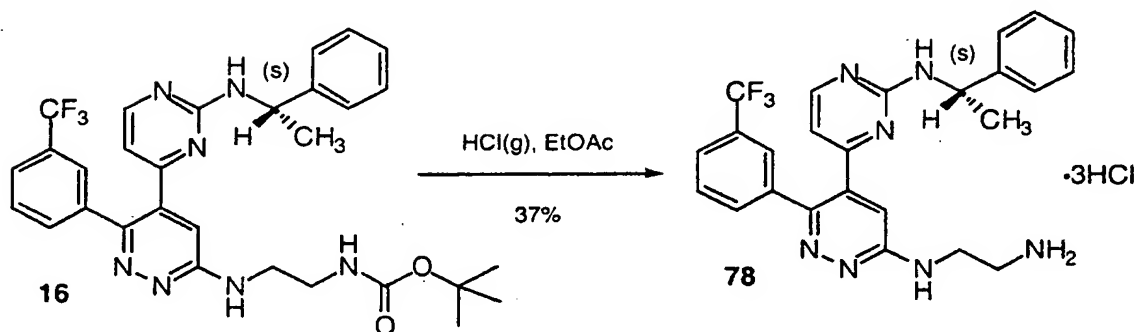
Scheme 2: Synthesis of Compound 73



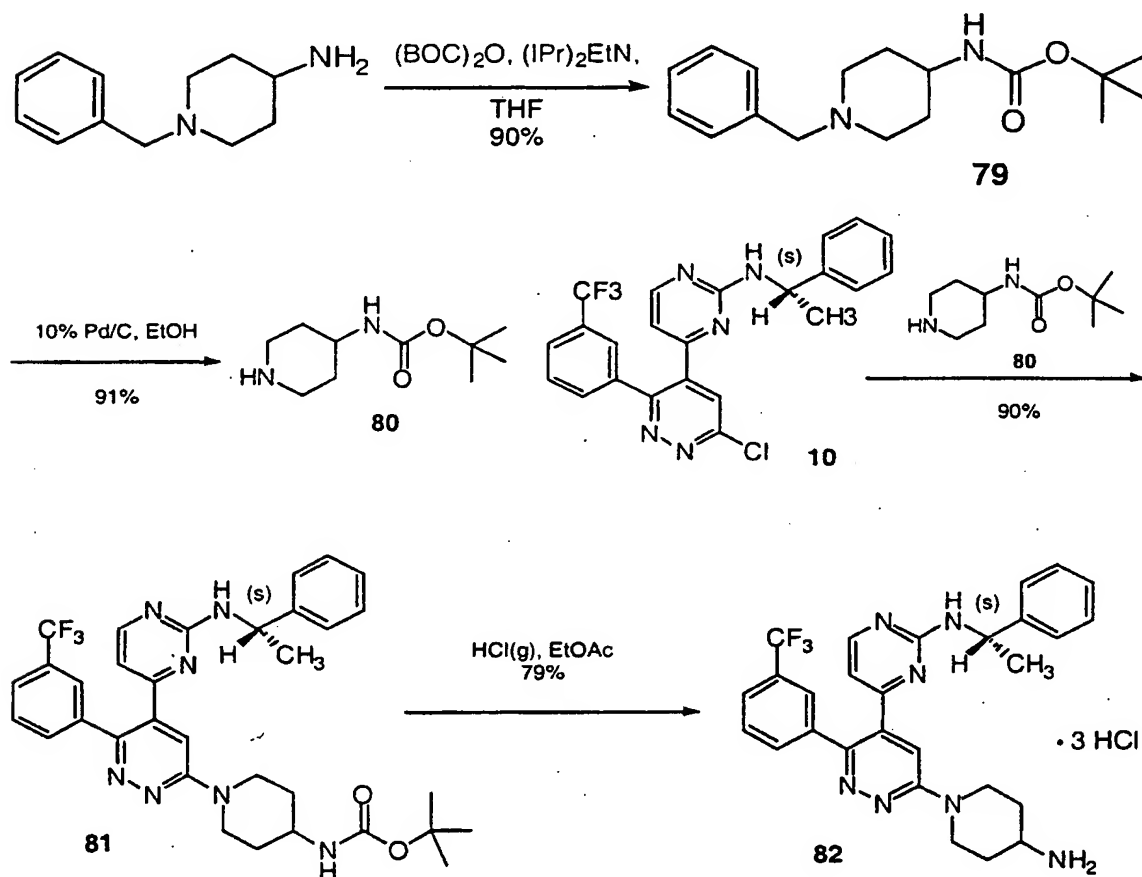
Scheme 3: Synthesis of Compound 77



Scheme 4: Synthesis of Compound 78

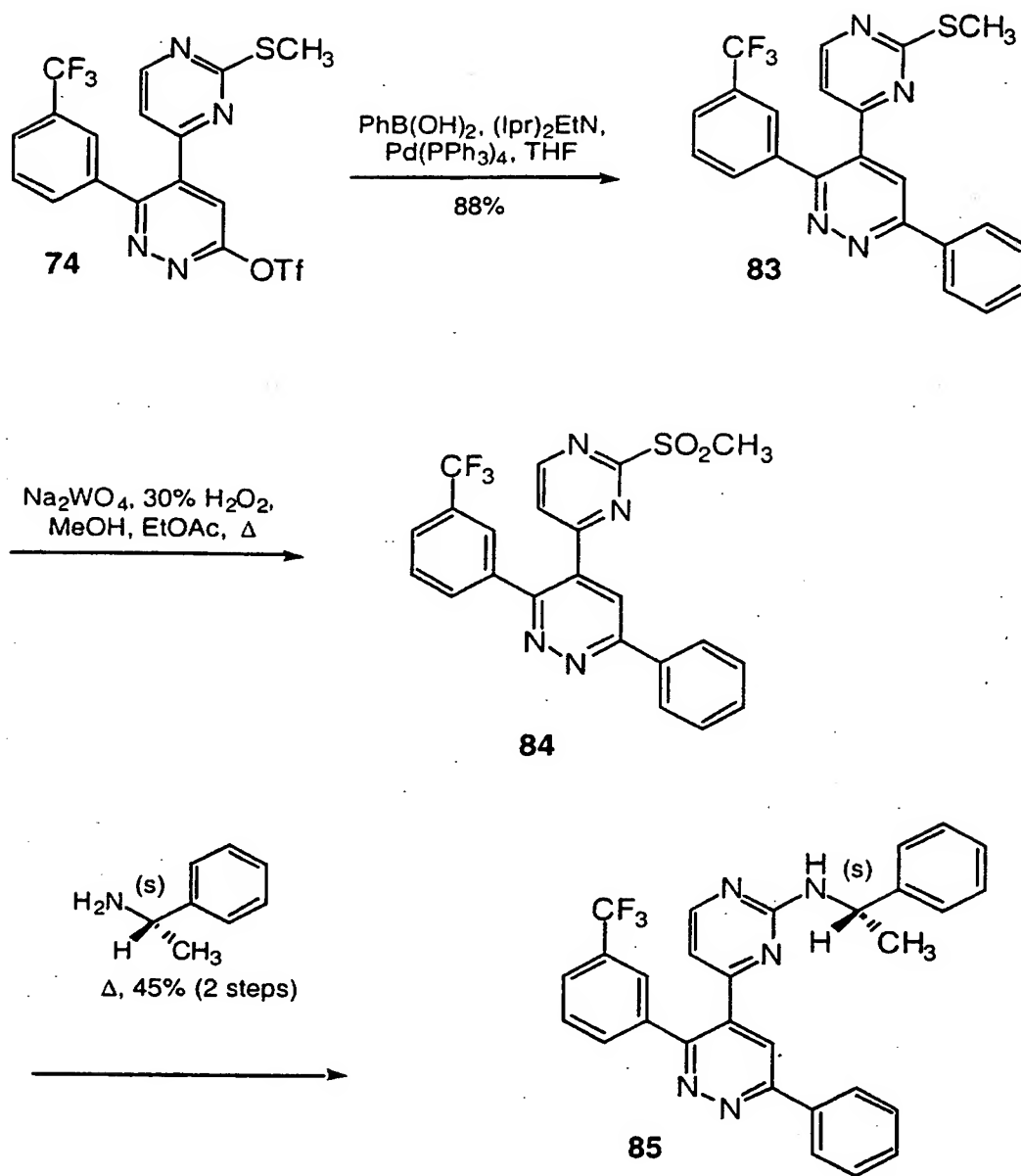


Scheme 5: Synthesis of Compound 82

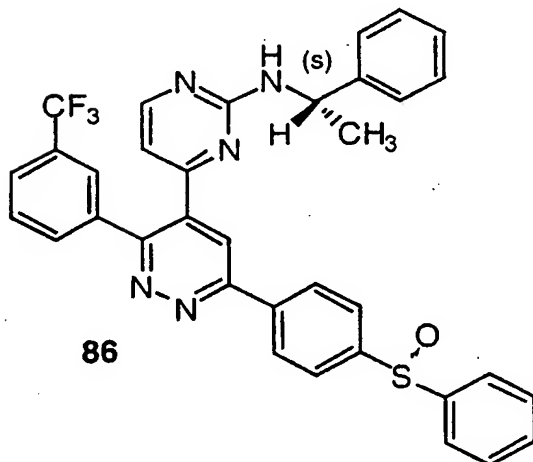
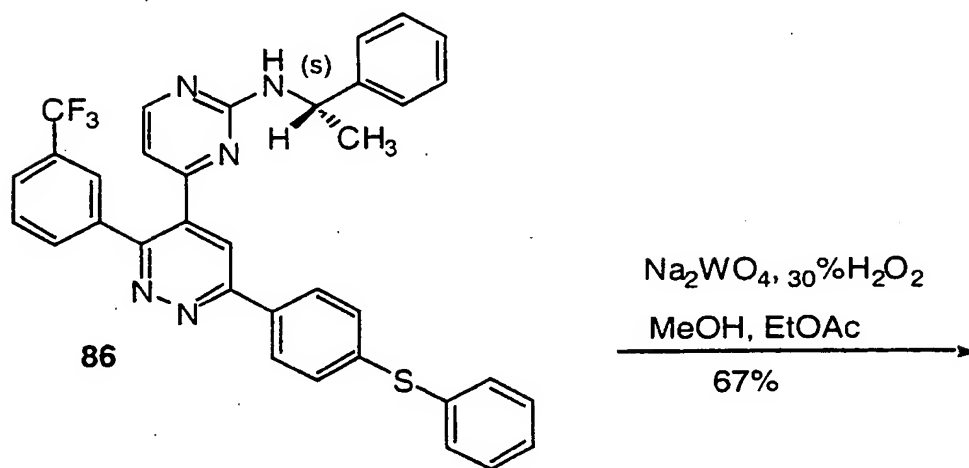
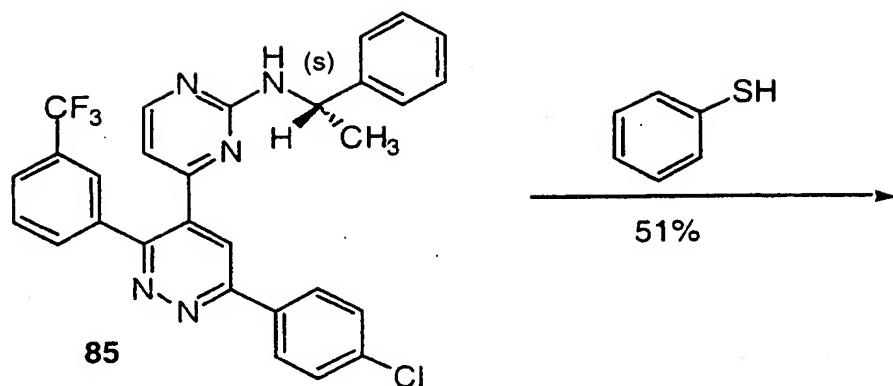


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Scheme 6



Scheme 7



The compounds of formula I can be used in the prophylactic or therapeutic treatment of disease states in mammals which are exacerbated or caused by excessive or unregulated cytokines, e.g., IL-1, IL-6, IL-8 or TNF.

Because the compounds of formula I inhibit cytokines, the compounds
5 are useful for treating diseases in which cytokine presence or activity is implicated, such as rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions.

The compounds of formula I are useful to treat disease states mediated by excessive or unregulated TNF production or activity. Such diseases include, but
10 are not limited to sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, such as osteoporosis, reperfusion injury, graft v. host rejection, allograft rejection, fever, myalgia due to infection, cachexia secondary to infection or
15 malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDs related complex), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, pyresis, AIDS and other viral infections, such as cytomegalovirus (CMV), influenza virus, and the herpes family of viruses such as Herpes Zoster or Simplex I and II.

20 The compounds of formula I are also useful topically in the treatment of inflammation such as in the treatment of rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions; inflamed joints, eczema, psoriasis or other inflammatory skin conditions such as sunburn; inflammatory eye conditions including conjunctivitis; pyresis, pain and other
25 conditions associated with inflammation.

The compounds of formula I are also useful in treating diseases characterized by excessive IL-8 activity. These disease states include psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis.

30 The invention thus includes a method of treating psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis, in a mammal in need of such treatment, which comprises administering to said mammal a compound of formula I in an amount which is effective for treating said disease or condition.

When administered to a patient for the treatment of a disease in which a cytokine or cytokines are implicated, the dosage used can be varied within wide limits, depending upon the type of disease, the age and general condition of the patient, the particular compound administered, the presence or level of toxicity or adverse effects experienced with the drug and other factors. A representative example of a suitable dosage range is from as low as about 0.01 mg/kg to as high as about 100 mg/kg. However, the dosage administered is generally left to the discretion of the physician.

The methods of treatment can be carried out by delivering the compound of formula I parenterally. The term 'parenteral' as used herein includes intravenous, intramuscular, or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. The instant invention can also be carried out by delivering the compound of formula I through subcutaneous, intranasal, intrarectal, transdermal or intravaginal routes.

The compounds of formula I may also be administered by inhalation. By 'inhalation' is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by convention techniques.

The invention also relates to a pharmaceutical composition comprising a compound of formula I and a pharmaceutically acceptable carrier. The compounds of formula I may also be included in pharmaceutical compositions in combination with a second therapeutically active compound.

The pharmaceutical carrier employed may be, for example, either a solid, liquid or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Examples of liquid carriers are syrup, peanut oil, olive oil, water and the like. Examples of gaseous carriers include carbon dioxide and nitrogen.

Similarly, the carrier or diluent may include time delay material well known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

A wide variety of pharmaceutical dosage forms can be employed. If a solid dosage is used for oral administration, the preparation can be in the form of a tablet, hard gelatin capsule, troche or lozenge. The amount of solid carrier will vary widely, but generally will be from about 0.025 mg to about 1 g. When a liquid dosage form is desired for oral administration, the preparation is typically in the form of a

syrup, emulsion, soft gelatin capsule, suspension or solution. When a parenteral dosage form is to be employed, the drug may be in solid or liquid form, and may be formulated for administration directly or may be suitable for reconstitution.

Topical dosage forms are also included. Examples of topical dosage forms are solids, liquids and semi-solids. Solids would include dusting powders, poultices and the like. Liquids include solutions, suspensions and emulsions. Semi-solids include creams, ointments, gels and the like.

The amount of a compound of formula I used topically will, of course, vary with the compound chosen, the nature and severity of the condition, and can be varied in accordance with the discretion of the physician. A representative, topical, dose of a compound of formula I is from as low as about 0.01 mg to as high as about 2.0 g, administered one to four, preferably one to two times daily.

The active ingredient may comprise, for topical administration, from about 0.001% to about 10% w/w.

Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container aseptically. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

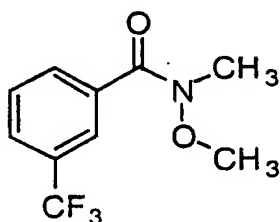
Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in

solution or suspension in an aqueous or non-aqueous liquid, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogels. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicas, and other ingredients such as lanolin may also be included.

The following examples illustrate the preparation of some of the compounds of the invention and are not to be construed as limiting the invention disclosed herein.

EXAMPLE 1

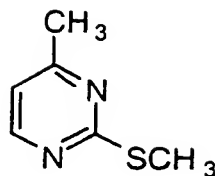


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N-Methoxy-N-methyl-3-trifluoromethylbenzamide (1)

3-(Trifluoromethyl)-benzoyl chloride (50mL, 332mmol), N,O-dimethyl-hydroxylamine hydrochloride (42g, 431mmol) and methylene chloride were combined under argon, then cooled in an ice bath. Triethylamine (108mL, 775mmol) was added dropwise with stirring over 0.5h. The contents of the reaction flask were then warmed to room temperature slowly. After 18h, the reaction suspension was washed successively with 5% potassium bisulfate, sat. sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate then concentrated *in vacuo* to give Compound 1 as an oil: 82g, (332mmol, 100%)

¹H NMR (CDCl₃, 300MHz) δ 7.98 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 3.55 (s, 3H), 3.39 (s, 3H).

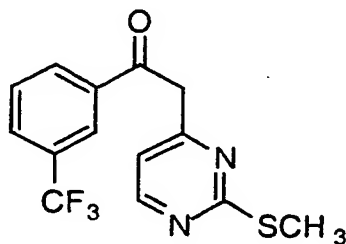


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4-Methyl-2-methylsulfanylpuridine (2)

5 2-Mercapto-4-methylpuridine hydrochloride (100g, 617mmol), dimethylformamide dimethylacetal (100mL, 754mmol), diisopropylethylamine (161 mL, 926mmol) and toluene (200mL) were combined under argon. The resulting solution was heated to reflux for 4h. The solvent was removed *in vacuo* and water and sodium bisulfate were added. The resulting mixture was extracted with ether
 10 (3 × 100mL). The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to afford an oil. Vacuum distillation gave Compound 2 as a liquid: 70.5g, (504mmol, 82%)

¹H NMR (CDCl₃, 300MHz) δ 8.36 (d, *J* = 5.1 Hz, 1H), 6.81 (d, *J* = 5.1 Hz, 1H), 2.56
 15 (s, 3H), 2.46 (s, 3H).



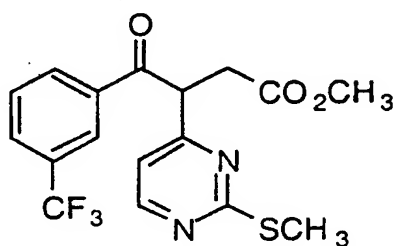
3

2-(2-Methylsulfanylpuridin-4-yl)-1-(3-trifluoromethylphenyl)ethanone (3)

20 Diisopropylamine (106mL, 761mmol) and tetrahydrofuran (THF) (750mL) were combined in a 3-neck 2L round bottom flask under argon. Two addition funnels, one containing a solution of Compound 1 in THF (100mL), the other containing Compound 2 in THF (100mL) were attached to the reaction flask. The contents of the reaction flask were cooled in an IPA/dry ice bath. A solution of

n-butyl lithium (2.5M in hexanes, 304mL, 761mmol) was added dropwise via syringe. The THF solution of Compound 1 was added dropwise over 0.5h. This was followed by the addition of the THF solution of Compound 2. A dark solution resulted. After 10 min, ice was added and the THF was removed *in vacuo*. Water was added followed by sat. sodium bicarbonate and the aqueous mixture was extracted several times with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give an oil. Purification by flash column chromatography (hexane:ethyl acetate 95:5 to 75:25) gave a solid which was triturated with hexane:ether 80:20 then isolated by vacuum filtration to afford Compound 3 as a solid: 60g (220mmol, 43%)

¹H NMR (CDCl₃, 300MHz) δ 14.7 (s, 1H), 8.36 (d, *J* = 5.1 Hz, 1H), 8.09 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 6.70 (d, *J* = 5.5 Hz, 1H), 6.04 (s, 1H), 2.62 (s, 3H).



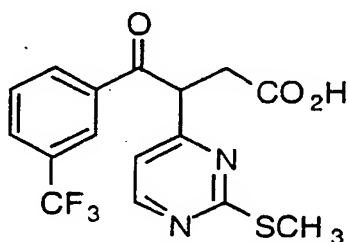
4

3-(2-Methylsulfanylpurin-4-yl)-4-oxo-4-(3-trifluoromethylphenyl)butyric acid methyl ester (4)

Sodium hydride (95%, 1.78g, 83mmol) and dimethyl sulfoxide (DMSO) (150mL) were combined under argon in a 3-neck 1L round bottom flask. A solution of Compound 3 in DMSO (50mL) was added dropwise over 0.75h using an addition funnel. After stirring an additional 0.5h, methyl bromoacetate (7.9mL, 83mmol) in DMSO (50mL) was added dropwise. After 18h, the contents of the reaction flask were poured into water. Saturated sodium bicarbonate was added and the resulting solution was extracted several times with ethyl acetate. The combined organic extracts were washed successively with water and brine. The organic layer was dried with anhydrous sodium sulfate then concentrated *in vacuo* to give an oil.

Purification by flash column chromatography (hexane:ethyl acetate 70:30) afforded Compound 4 as an oil: 24.6g (64mmol, 100%)

¹H NMR (CDCl₃, 300MHz) δ 8.42 (d, *J* = 5.2 Hz, 1H), 8.33 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 6.89 (d, *J* = 5.2 Hz, 1H), 5.30-5.21 (m, 1H), 3.69 (s, 3H), 3.49-3.40 (m, 1H), 2.88 (dd, *J* = 17.2, 4.7 Hz, 1H), 2.49 (s, 3H).



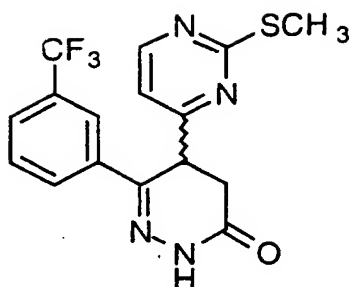
10

5

3-(2-Methylsulfanylpirimidin-4-yl)-4-oxo-4-(3-(trifluoromethyl)phenyl)butyric acid (5)

A solution of Compound 4 (27.1g, 64mmol) and dioxane (500mL) were stirred under argon. Hydrochloric acid (6N, 250mL) was added dropwise using an addition funnel. After 3d dioxane was removed *in vacuo*. The remaining aqueous mixture was extracted exhaustively with methylene chloride. The combined organic
15 extracts were dried over anhydrous sodium sulfate then the solvent was removed *in vacuo* to give a yellow oil. Hexane followed by ether was added to afford a solid (5) which was isolated by vacuum filtration: 18.6g (50.4mmol, 79%)

20 ¹H NMR (CDCl₃, 300MHz) δ 8.42 (d, *J* = 5.2 Hz, 1H), 8.33 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 6.89 (d, *J* = 5.2 Hz, 1H), 5.30-5.21 (m, 1H), 3.69 (s, 3H), 3.49-3.40 (m, 1H), 2.88 (dd, *J* = 17.2, 4.7 Hz, 1H), 2.49 (s, 3H).

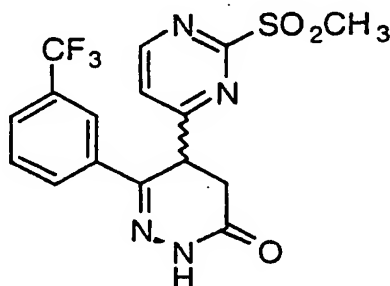


6

5-(2-Methylsulfanylpirimidin-4-yl)-6-(3-trifluoromethylphenyl)-4,5-dihydro-2H-pyridazin-3-one (6)

5 Compound 5 (6.58g, 17.8mmol), and ethanol (200mL) were combined under argon. Hydrazine (835μL, 26.6mmol) was added and the resulting solution was heated at reflux for 4h. The solution was cooled and rotary evaporated. Ethyl acetate was added followed by the addition of ether. The solution was again rotary evaporated to afford a foam (6) which was used as is for the synthesis of 7.

10



7

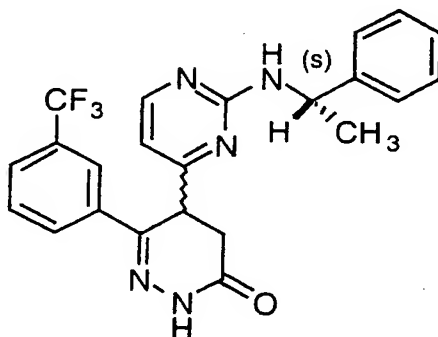
5-(2-Methylsulfonylpirimidin-4-yl)-6-(3-trifluoromethylphenyl)-4,5-dihydro-2H-pyridazin-3-one (7)

15 Compound 6 (17.8mmol), sodium tungstate (587mg, 1.78mmol), 30% hydrogen peroxide (8.07mL, 71.2mmol), methanol (20mL), and ethyl acetate (200mL) were combined under argon, then heated to reflux for 4h. The solution was cooled and aqueous sodium hydrosulfite was added. The methanol was removed *in vacuo* and saturated sodium bicarbonate was added. The resulting suspension was extracted

20 several times with methylene chloride. The combined organic extracts were washed successively with water and brine. The organic layer was dried with anhydrous

sodium sulfate then concentrated *in vacuo* to give Compound 7 as a foam: 5.0g (13mmol, 73% (two steps))

¹H NMR (CDCl₃, 300MHz) δ 8.85 (d, *J* = 5.2 Hz, 1H), 8.79 (s, 1H), 8.11 (s, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 5.2 Hz, 1H), 4.82-4.78 (m, 1H), 3.33 (s, 3H), 3.17-2.05 (m, 2H).

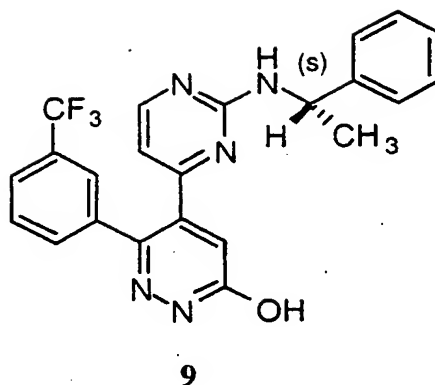


8

10 (s)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-6-(3-trifluoromethylphenyl)-4,5-dihydro-2H-pyridazin-3-one (8)

Compound 7 (5.00g, 13.1mmol), *s*-(-)-α-methylbenzylamine (3.37mL, 26.2mmol) and tetrahydrofuran (100mL) were heated to reflux under argon for 24h. The solvent was removed *in vacuo*. Purification by flash column chromatography (methylene chloride:methanol: ammonium hydroxide 98:2:0.2) gave Compound 8 as
15 a foam: 5.0g (9.6mmol, 73%).

¹H NMR (CDCl₃, 300MHz) δ 8.62-8.50 (m, 1H), 8.18-8.12 (m, 1H), 8.10-8.00 (m, 1H), 7.80 (d, *J* = 2.7 Hz, 1H), 7.60 (m, 1H), 7.52-7.44 (m, 1H), 7.38-7.20 (m, 5H), 6.33 (t, *J* = 4.3 Hz, 1H), 5.50 (m, 1H), 5.10-5.00 (m, 1H), 4.40-4.30 (m, 1H), 3.10-2.80 (m, 2H), 1.51 (d, *J* = 6.7 Hz, 3H).

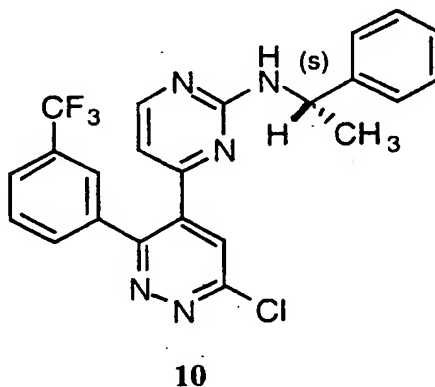


(s)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-6-(3-trifluoromethylphenyl)-pyridazin-3-ol (9)

- 5 Compound 8 (4.24g, 9.66mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (4.38g, 19.3mmol), and acetonitrile (100mL) were combined and stirred at room temperature under argon for 24h. The solvent was removed *in vacuo* and 5% ammonium hydroxide was added. The solution was extracted exhaustively with methylene chloride. The combined organic extracts were dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* to give a solid. Trituration with
- 10 hexane-ether gave Compound 9 as a solid: 3.5g (8.0mmol, 82%)

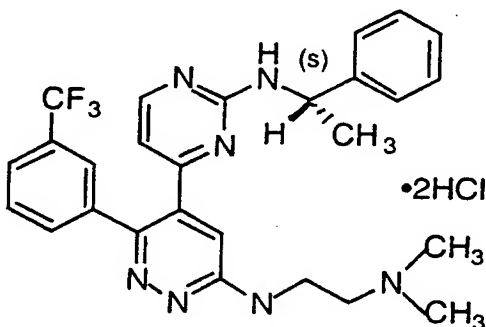
¹H NMR (CDCl₃, 300MHz) δ 12.20-12.00 (s, br, 1H), 8.24 (d, *J* = 4.9 Hz, 1H), 7.64-7.56 (m, 2H), 7.42-7.20 (m, 8H), 6.40-6.34 (m, 1H), 5.80 (s, br, 1H), 4.80 (s, br, 1H),

15 1.25 (m, br, 3H).



- 20 (s)-6-Chloro-4-[2-(1-phenylethylamino)pyrimidin-4-yl]-3-(3-trifluoromethylphenyl)pyridazine (10)

- Compound 9 (3.5g, 7.96mmol) and phosphorus oxychloride (20mL) were combined under argon then heated at 90°C for 0.5h. The solution was cooled and poured onto ice. The resulting suspension was made basic with 2N sodium hydroxide and extracted several times with methylene chloride. The combined organic extracts were washed with brine and dried with anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* gave an oil which was purified by flash column chromatography (hexane ethyl acetate 80:20) to give Compound 10 as a foam: 2.9g (6.4mmol, 80%)
- ¹H NMR (CDCl₃, 300MHz) δ 8.16 (d, *J* = 4.9Hz, 1H), 7.83 (s, 1H), 7.72 - 7.45 (m, 4H), 7.40 - 7.25 (m, 5H), 6.21 (d, *J* = 4.9Hz, 1H), 5.65 - 5.55 (s, br. 1H), 5.05 - 4.90 (s, br. 1H), 1.51 (d, *J* = 7.0 Hz, 3H).



15

11

(s)-N-1-[5-[2-(1-phenethyl)aminopyrimidin-4-yl]-6-(3-trifluoromethylphenyl)pyridazin-3-yl]-N-2-(dimethyl)ethane-1,2-diamine dihydrochloride (11)

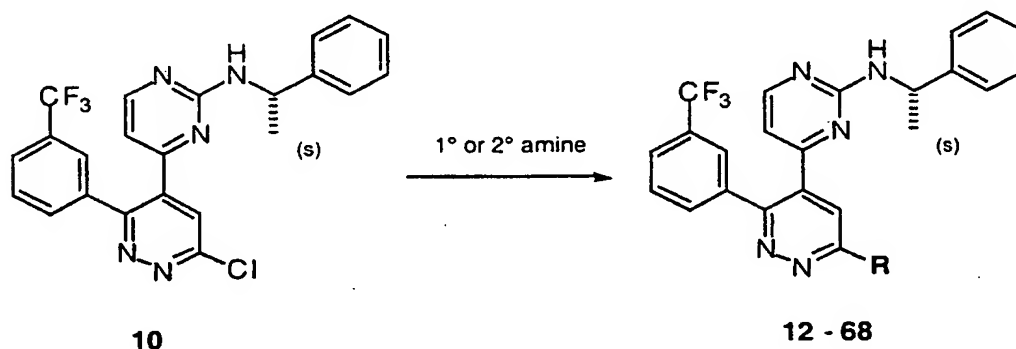
- Compound 10 (100 mg, 0.220mmol), and dimethylethylenediamine (~500μL) were combined under argon and heated at 100°C for 1h. The contents of the reaction flask were poured into saturated sodium bicarbonate and extracted several times with methylene chloride. The combined organic portions were dried with anhydrous sodium sulfate and concentrated *in vacuo*. Purification by flash column chromatography (methylene chloride:methanol:ammonia 95:5:0.5) gave a foam which was dissolved in ether. Addition of 1N hydrogen chloride in ether gave Compound 11 as a solid: 8mg (0.152mmol, 69%)

¹H NMR (CD₃OD, 300MHz) δ 8.42-8.32 (m, 1H), 7.84-7.68 (m, 3H), 7.66-7.58

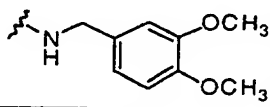
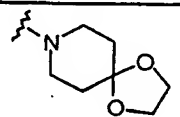
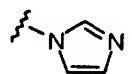
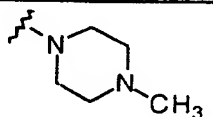
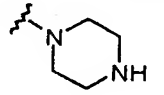
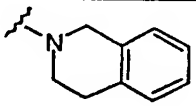
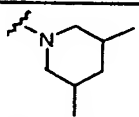
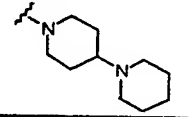
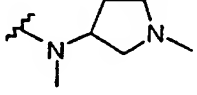
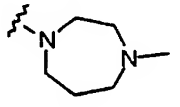
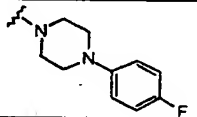
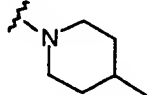
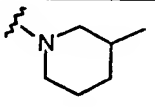
(m, 2H), 7.32-7.16 (m, 5H), 6.96-6.80 (s, br, 1H), 3.99 (t, $J = 6.0\text{Hz}$, 2H), 3.54 (t, $J = 6.0\text{Hz}$, 2H), 3.01 (s, 6H), 1.45-1.25 (s, br, 3H).

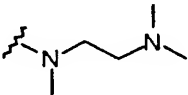
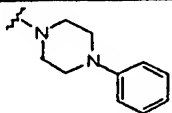
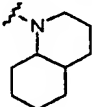
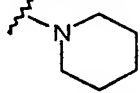
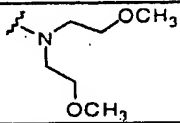
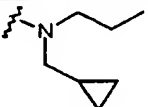
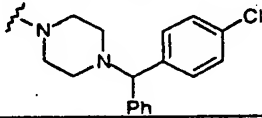
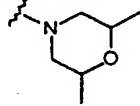
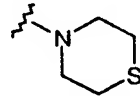
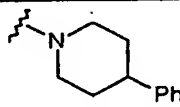
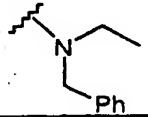
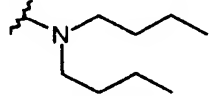
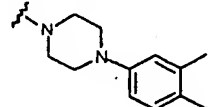
Compounds **12 - 68** were prepared in a manner similar to that described for the preparation of **11**. Mass spectrometry was used to characterize either the free base, hydrochloride salt, or trifluoroacetic acid salt of compounds **12 - 68**. These compounds are summarized below in Table 1.

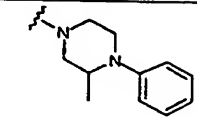
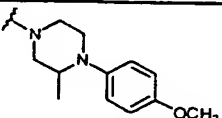
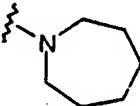

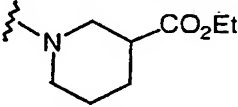
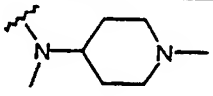
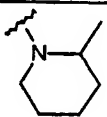
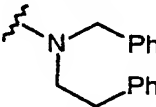
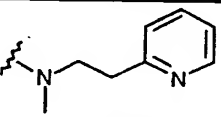
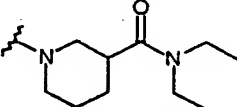
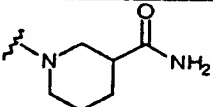
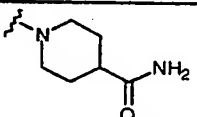
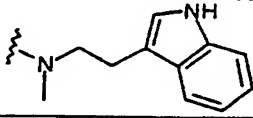
Table 1: Reaction of **10** With 1° or 2° Amines

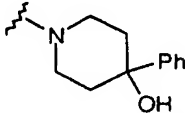
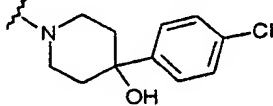
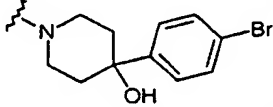
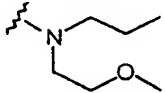
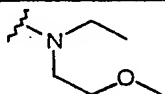
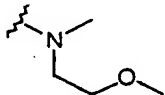
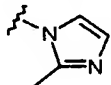
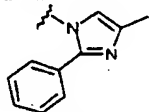
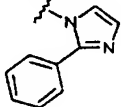
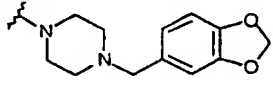
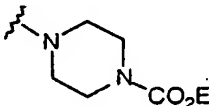
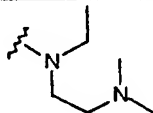


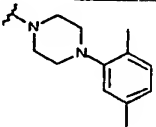
| Number | R | MS $[M + H]^+$ | Salt Form |
|--------|---|----------------|-----------|
| 12 | | 509.3 | free base |
| 13 | | 523.2 | free base |
| 14 | | 546.3 | free base |
| 15 | | 610.2 | free base |
| 16 | | 581.2 | • 2 HCl |

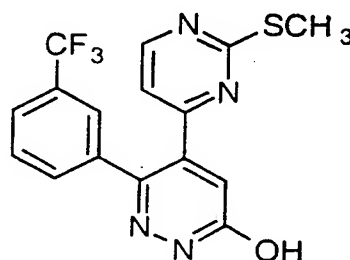
| | | | |
|----|---|-------|-----------|
| 17 |  | 587.0 | • 2 HCl |
| 18 |  | 563.0 | free base |
| 19 |  | 488.3 | free base |
| 20 |  | 520.2 | • 3 HCl |
| 21 |  | 506.1 | • 3 HCl |
| 22 |  | 553.5 | • 2 TFA |
| 23 |  | 533.3 | • 2 TFA |
| 24 |  | 588.0 | • 3 TFA |
| 25 |  | 535.4 | • 3 TFA |
| 26 |  | 534.1 | • 3 TFA |
| 27 |  | 600.2 | • 3 TFA |
| 28 |  | 519.1 | • 2 TFA |
| 29 |  | 519.2 | • 2 TFA |

| | | | |
|----|---|-------|---------|
| 30 |  | 522.2 | • 3 TFA |
| 31 |  | 582.5 | • 3 TFA |
| 32 |  | 559.4 | • 2 TFA |
| 33 |  | 505.5 | • 1 HCl |
| 34 |  | 553.5 | • 2 TFA |
| 35 |  | 533.3 | • 2 TFA |
| 36 |  | 708.6 | • 3 TFA |
| 37 |  | 535.2 | • 2 TFA |
| 38 |  | 523.2 | • 2 TFA |
| 39 |  | 581.2 | • 2 TFA |
| 40 |  | 555.2 | • 2 TFA |
| 41 |  | 549.3 | • 2 TFA |
| 42 |  | 610.2 | • 3 TFA |

| | | | |
|----|---|-------|---------|
| 43 |  | 596.4 | • 3 TFA |
| 44 |  | 626.2 | • 3 TFA |
| 45 |  | 519.3 | • 2 TFA |
| 46 |  | 489.2 | • 2 TFA |
| 47 |  | 577.4 | • 2 TFA |
| 48 |  | 548.3 | • 3 TFA |
| 49 |  | 519.5 | • 2 TFA |
| 50 |  | 631.0 | • 2 TFA |
| 51 |  | 556.2 | • 2 TFA |
| 52 |  | 604.4 | • 2 TFA |
| 53 |  | 548.4 | • 2 TFA |
| 54 |  | 548.2 | • 2 TFA |
| 55 |  | 594.2 | • 2 TFA |

| | | | |
|----|---|-------|---------|
| 56 |  | 597.4 | • 2 TFA |
| 57 |  | 631.2 | • 2 TFA |
| 58 |  | 677.4 | • 2 TFA |
| 59 |  | 537.9 | • 2 TFA |
| 60 |  | 523.3 | • 2 TFA |
| 61 |  | 509.0 | • 2 TFA |
| 62 |  | 502.0 | • 1 TFA |
| 63 |  | 578.8 | • 1 TFA |
| 64 |  | 563.9 | • 1 TFA |
| 65 |  | 640.2 | • 3 TFA |
| 66 |  | 578.5 | • 2 TFA |
| 67 |  | 535.9 | • 3 TFA |

| | | | |
|----|---|-------|---------|
| 68 |  | 611.0 | • 3 TFA |
|----|---|-------|---------|

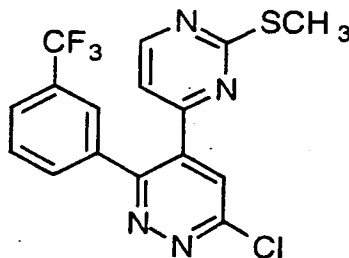
EXAMPLE 2

69

5-(2-Methylsulfanylpirimidin-4-yl)-6-(3-(trifluoromethyl)phenyl)pyridazin-3-ol (69)

Compound **6** (1.92g, 5.26mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (4.78g, 21.0mmol), and acetonitrile (30mL) were combined under argon and heated at 90°C for 1h. The solution was cooled and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (methylene chloride:methanol:ammonium hydroxide 95:5:0.5) to give a solid. Trituration with Et₂O/EtOAc gave Compound **69**: 1.60g (4.4mmol, 83%)

¹H NMR (CDCl₃, 300MHz) δ 12.95 (s, 1H), 8.51 (d, *J* = 4.9 Hz, 1H), 7.67-7.61 (m, 2H), 7.46-7.39 (m, 1H), 7.34-7.27 (m, 2H), 6.84 (d, *J* = 5.2 Hz, 1H), 2.25 (s, 3H).

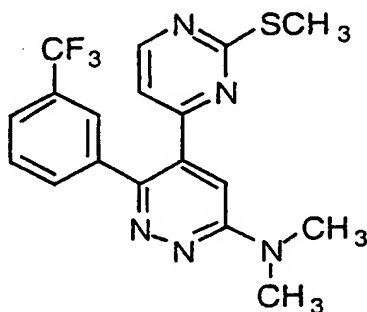


70

6-Chloro-4-(2-methylsulfanylethylpyrimidin-4-yl)-3-(3-trifluoromethylphenyl)-pyridazine (70)

Compound **69** (1.32g, 3.63mmol) and phosphorus oxychloride (10mL) were combined under argon and heated at 90°C for 1h. The solution was cooled and excess phosphorus oxychloride was removed *in vacuo*. Water was added followed by sat. sodium bicarbonate and the solution was extracted several times with ethyl acetate. The combined organic extracts were washed with brine and dried with anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* gave a solid. Purification by flash column chromatography (hexane ethyl acetate 70:30) gave an oil which was crystallized using hexane:ethyl acetate 90:10 to give Compound **70**: 937mg (2.45mmol, 67%)

¹H NMR (CDCl₃, 300MHz) δ 8.45 (d, *J* = 4.9Hz, 1H), 7.91 (s, 1H), 7.86 (s, 1H), 7.76-7.70 (m, 1H), 7.58-7.48 (m, 2H), 6.69 (d, *J* = 5.2Hz, 1H), 2.45 (s, 3H).

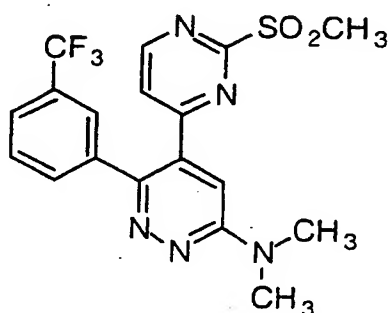


71

Dimethyl-[5-(2-methylsulfanylpurine-4-yl)-6-(3-trifluoromethylphenyl)-pyridazin-3-yl]amine (71)

Compound **70** (200mg, 0.522mmol) and ethanol (4mL) were combined in a threaded glass pressure tube. The reaction solution was cooled in an ice bath and dimethylamine was bubbled through the solution until saturated. The solution was sealed and heated to 60°C for 24h. The solvent was removed *in vacuo*. Purification by flash column chromatography (hexane:ethyl acetate 60:40) gave an oil which crystallized from hexane: ethyl acetate to afford Compound **71**: 128mg (0.327mmol, 63%)

¹H NMR (CDCl₃, 300MHz) δ 8.39 (d, *J* = 5.2Hz, 1H), 7.85 (s, 1H), 7.58 (d, *J* = 7.3Hz, 1H), 7.48-7.36 (m, 2H), 7.07 (s, 1H), 6.67 (d, *J* = 5.2Hz, 1H), 3.30 (s, 6H), 2.45 (s, 3H).

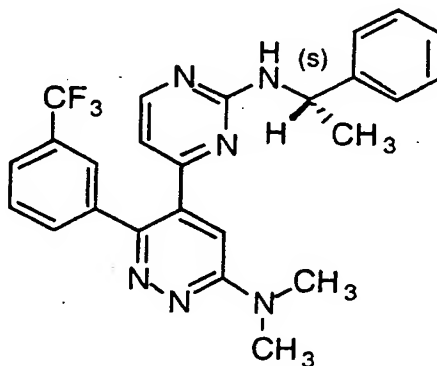


72

Dimethyl-[5-(2-methylsulfonylpyrimidin-4-yl)-6-(3-trifluoromethylphenyl)-pyridazin-3-yl]amine (72)

Compound 71 (112mg, 0.286mmol), sodium tungstate (10mg, 0.02 mmol), 30% hydrogen peroxide (130μL, 1.14mmol), methanol (2.0mL), and ethyl acetate (20mL) were combined under Argon and heated to reflux for 3d. The solution was cooled, poured into water and extracted several times with ethyl acetate. The combined organic extracts were dried with anhydrous sodium sulfate. Removal of the solvent *in vacuo* gave 72 as a solid: 109mg (0.259mmol, 91%)

¹H NMR (CDCl₃, 300MHz) δ 8.77 (d, *J* = 5.2Hz, 1H), 7.82 (s, 1H), 7.66-7.60 (m, 1H), 7.48-7.42 (m, 2H), 7.24-7.20 (m, 1H), 7.15 (s, 1H), 3.33 (s, 6H), 3.27 (s, 3H).



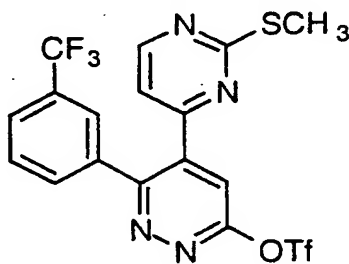
73

(s)-Dimethyl-{5-[2-(1-phenethyl)aminopyrimidin-4-yl]-6-(3-trifluoromethyl-phenyl)pyridazin-3-yl}amine (73)

Compound 72 (155mg, 0.367mmol) and s-(-)- α -methylbenzylamine (0.500mL) were combined under Argon and heated at 100°C for 0.5h. The solution was cooled and purified by flash column chromatography (hexane:ethyl acetate 50:50) to give an oil which was crystallized using hexane ethyl acetate ether to give Compound 73: 80mg (0.172mmol, 47%)

¹H NMR (CDCl₃, 300MHz) δ 8.12 (d, J = 5.2Hz, 1H), 7.83 (s, 1H), 7.54 (t, J = 9.0Hz, 2H), 7.40-7.20 (m, 6H), 6.85 (s, 1H), 6.21 (d, J = 4.9Hz, 1H), 5.51 (d, J = 7.3Hz, 1H), 5.10-5.00 (s, br, 1H), 3.22 (s, 6H), 1.51 (d, J = 6.7Hz, 3H).

EXAMPLE 3

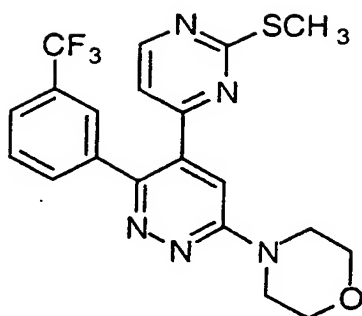


74

Trifluoromethanesulfonic acid 5-(2-methylsulfanylpurimidine-4-yl)-6-(3-trifluoromethylphenyl)pyridazin-3-yl ester (74)

Compound 69 (2.59g, 7.11mmol), and methylene chloride (100mL) were combined under argon and cooled in an ice bath. Diisopropylethylamine (1.61mL, 9.24mmol) was added followed by trifluoromethanesulfonic anhydride (1.58mL, 9.24mmol). After 1h, the solvent was removed *in vacuo*. Flash column chromatography (hexane: ethyl acetate 80:20) followed by trituration with hexane and ether afforded Compound 74 as a solid: 3.15g (6.37mmol, 89%)

¹H NMR (CDCl₃, 300MHz) δ 8.48 (d, J = 4.9Hz, 1H), 7.90 (s, 1H), 7.84 (s, 1H), 7.80-7.74 (m, 1H), 7.62-7.50 (m, 2H), 6.70 (d, J = 5.2Hz, 1H), 2.47 (s, 3H).

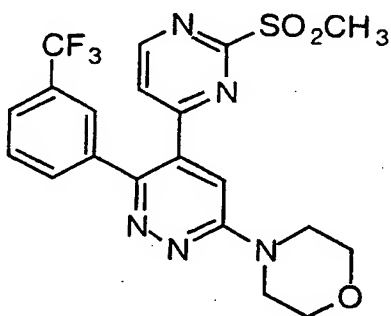


75

4-[5-(2-methylsulfonylpyrimidin-4-yl)-6-(3-trifluoromethylphenyl)-pyridazin-3-yl]morpholine (75)

5 Compound 74 (180mg, 0.363mmol) and morpholine (~1mL) were combined under Argon and heated to 80°C for 2h. Purification by flash column chromatography (hexane ethyl acetate 50:50) gave Compound 75 as an oil: 104mg (0.240mmol, 66%)

10 ¹H NMR (CDCl₃, 300MHz) δ 8.39 (d, *J* = 5.2Hz, 1H), 7.86 (s, 1H), 7.64-7.59 (m, 1H), 7.50-7.38 (m, 2H), 7.20 (s, 1H), 6.65 (d, *J* = 4.9Hz, 1H), 3.95-3.85 (m, 4H), 3.85-3.75 (m, 4H), 2.46 (s, 3H).



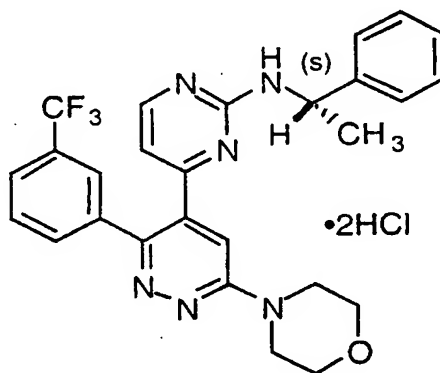
76

4-[5-(2-Methylsulfonylpyrimidin-4-yl)-6-(3-trifluoromethylphenyl)-pyridazin-3-yl]morpholine (76)

15 Compound 75 (104mg, 0.240mmol), sodium tungstate (7mg, 0.02mmol), 30% hydrogen peroxide (109μL, 0.960mmol), methanol (500μL), and
20 ethyl acetate (5mL) were combined under Argon and heated to reflux for 3d. The

solvent was removed *in vacuo* followed by flash column chromatography (ethyl acetate hexane 90:10) to afford Compound **76** as a solid: 75mg (0.161mmol, 67%)

¹H NMR (CDCl₃, 300MHz) δ 8.75 (d, *J* = 5.2Hz, 1H), 7.82 (s, 1H), 7.70-7.62 (m, 1H), 7.50-7.42 (m, 2H), 7.32-7.24 (m, 1H), 7.17 (d, *J* = 5.2Hz, 1H), 3.95-3.85 (m, 4H), 3.85-3.75 (m, 4H), 3.31 (s, 3H).



77

(s)-4-{5-[2-(1-Phenethylamino)pyrimidin-4-yl]-6-(3-trifluoromethylphenyl)pyridazin-3-yl}morpholine dihydrochloride (77)

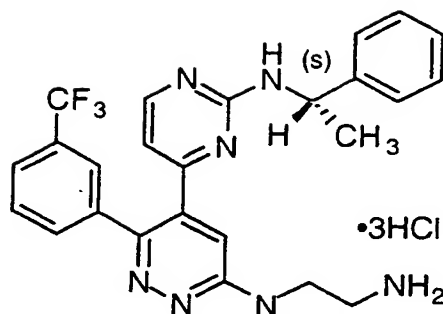
Compound **76** (75mg, 0.161mmol) and s-(-)-α-methylbenzylamine (500μL) were combined under Argon and heated at 100°C for 0.5h. Flash column chromatography (hexane:ethyl acetate 70:30) of the reaction mixture gave a mixture of the desired product and s-(-)-α-methylbenzylamine. Ethyl acetate was added and the mixture was washed with 5% potassium bisulfate. The organic layer was dried with anhydrous sodium sulfate and the solvent was removed *in vacuo* to give a foam. Treatment with 1N hydrogen chloride/ether gave Compound **77** as a solid: 33mg (0.057mmol, 35%)

20

¹H NMR (CD₃OD, 300MHz) δ 8.46-8.34 (s, br, 1H), 8.14-8.04 (s, br, 1H), 7.84-7.74 (m, 2H), 7.66-7.58 (m, 2H), 7.32-7.14 (m, 5H), 7.02-6.88 (s, br, 1H), 4.60-4.40 (s, br, 1H), 3.95-3.80 (m, 8H), 1.45-1.30 (s, br, 3H).

25

EXAMPLE 4



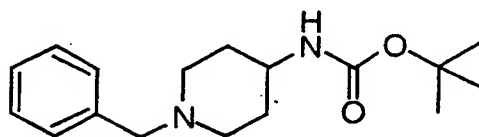
78

(s)-N-1-[5-[2-(1-phenethyl)aminopyrimidin-4-yl]-6-(3-trifluoromethylphenyl)-pyridazin-3-yl]ethane-1,2-diamine trihydrochloride (78)

Compound **16** (210mg, 0.363mmol) and ethyl acetate (15mL) were combined under Argon and cooled in an ice bath. The solution was saturated with hydrogen chloride gas. The mixture was stirred with cooling for 0.5h and warmed to room temperature for 1h. Removal of the solvent *in vacuo* gave a solid which was triturated with acetonitrile to afford 80mg (0.136mmol, 37%) of Compound **31**:

¹H NMR (CD₃OD, 300MHz) δ 8.38-8.30 (m, 1H), 8.22-8.16 (m, 1H), 7.94-7.86 (m, 2H), 7.80-7.70 (m, 2H), 7.66-7.50 (m, 5H), 7.32-7.18 (m, 5H), 6.52-6.44 (s, br, 1H), 1.44-1.36 (m, 3H).

EXAMPLE 5



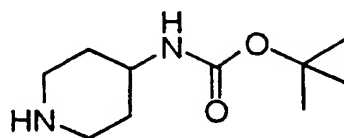
79

1-Benzyl-4-(tert-butoxycarbonylamino)piperidine (79)

4-Amino-1-benzylpiperidine (5.0mL, 24.6mmol) tetrahydrofuran (70mL), and diisopropylethylamine (10.3mL, 59.1mmol) were combined under Ar and cooled in an ice bath. Di-*tert*-butyl dicarbonate (6.45g, 29.6mmol) in tetrahydrofuran (30mL) was added dropwise. The reaction solution was allowed to warm to room temperature and stirred 24h. The solvent was removed *in vacuo* and

the remaining residue was purified by flash column chromatography (hexane:ethyl acetate 70:30) to give a solid. Trituration with ether gave 6.44g (22.2mmol, 90.3%) of Compound **79** as a solid.

- 5 ¹H NMR (CDCl₃, 300MHz) δ 7.30-7.26 (m, 5H), 4.45-4.35 (s, br, 1H), 3.48 (s, 3H), 2.85-2.75 (m, 2H), 2.15-2.05 (m, 2H), 1.95-1.85 (m, 2H), 1.44 (s, 11H).



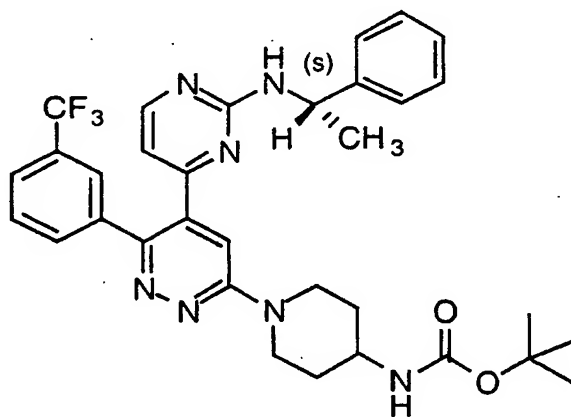
80

- 10 4-(tert-Butoxycarbonylamino)piperidine (80)

Compound **79** (4.55g, 15.7mmol), 10% palladium on carbon (400mg), and ethanol (100mL) were combined and hydrogenated in a Parr jar at 63psi for 3d. The mixture was filtrated through celite, followed by removal of the solvent *in vacuo* to afford 2.87g (14.4mmol, 91.4%) of Compound **80**.

15

¹H NMR (CDCl₃, 300MHz) δ 4.52-4.40 (s, br, 1H), 3.60-3.45 (s, br, 1H), 3.10-3.00 (m, 2H), 2.72-2.57 (m, 2H), 2.00-1.88 (m, 2H), 1.45 (s, 9H), 1.35-1.18 (m, 2H).



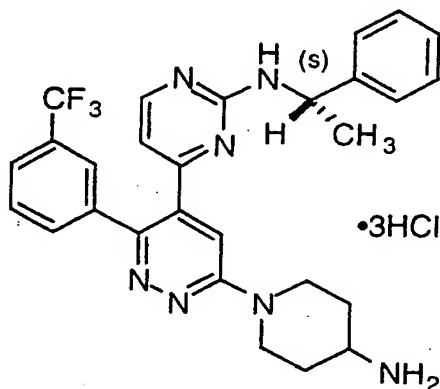
81

20

(s)-1-{5-[2-(1-Phenethylamino)pyrimidin-4-yl]-6-(3-trifluoromethylphenyl)-pyridazin-3-yl}-4-(tert-Butoxycarbonylamino)piperidine (81)

Compound **10** (320mg, 0.701mmol), Compound **80** (421mg, 2.10 mmol), and toluene (2mL) were combined under Argon and heated to 110°C for 8h. The contents of the reaction flask were poured into 5% potassium bisulfate and extracted several times with methylene chloride. The combined organic extracts were washed with brine and dried with anhydrous sodium sulfate. Purification by flash column chromatography (hexane:ethyl acetate 50:50) afforded 391mg (0.632mmol, 90%) of Compound **81** as a foam.

¹H NMR (CDCl₃, 300MHz) δ 8.11 (d, *J* = 5.2Hz, 1H), 7.83 (s, 1H), 7.60-7.50 (m, 2H), 7.42-7.22 (m, 6H), 6.96-6.88 (s, br, 1H), 6.17 (d, *J* = 4.9Hz, 1H), 5.53 (d, *J* = 7.6Hz, 1H), 5.10-5.00 (s, br, 1H), 4.54-4.30 (m, 3H), 3.86-3.70 (m, 1H), 3.20-3.08 (m, 2H), 2.14-2.04 (m, 2H), 1.60-1.44 (m, 12H), 1.30-1.24 (m, 2H).



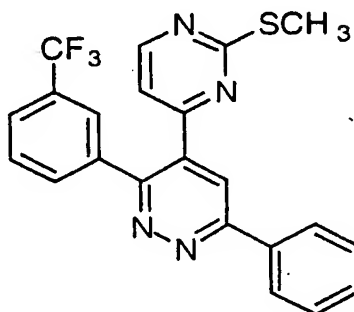
82

(s)-1-{5-[2-(1-Phenethylamino)pyrimidin-4-yl]-6-(3-trifluoromethylphenyl)-pyridazin-3-yl}-4-aminopiperidine trihydrochloride (82)

Compound **81** (391mg, 0.632mmol) and ethyl acetate (20mL) were combined under Ar and cooled in an ice bath. The solution was saturated with hydrogen chloride gas. An oil appeared in the reaction flask. Addition of ethanol (5mL) gave a solution. The solution was stirred for 1h and the solvent was removed *in vacuo*. The remaining residue was triturated with ethyl acetate to afford 313mg (0.498mmol, 79%) of Compound **82** as a solid.

¹H NMR (CD₃OD, 300MHz) δ 8.56-8.48 (s, br, 1H), 8.37 (s, 1H), 7.88-7.79 (m, 2H), 7.70-7.64 (m, 2H), 7.36-7.16 (m, 6H), 4.65-4.55 (m, 3H), 3.70-3.45 (m, 3H), 2.35-2.25 (m, 2H), 2.05-1.85 (m, 2H), 1.45-1.35 (m, 3H).

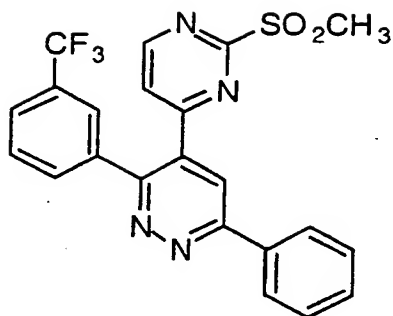
5

EXAMPLE 6

83

4-(2-Methylsulfonylpyrimidin-4-yl)-6-phenyl-3-(3-(trifluoromethyl)phenyl)-pyridazine

- 10 Compound 74 (112mg, 0.23mmol), phenylboronic acid (33mg, 0.27 mmol), diisopropylethylamine (141μL, 0.81mmol), tetrakis(triphenylphosphine)-palladium(0) (26mg, 0.023mmol), and tetrahydrofuran (2mL) were combined under argon and heated at reflux for 24h. The mixture was cooled and poured into water and sat. sodium bicarbonate. The resultant solution was extracted several times with
- 15 ether. The combined organic extracts were dried with anhydrous sodium sulfate and the solvent was removed *in vacuo*. Purification by flash column chromatography (hexane:ethyl acetate 80:20 then 60:40) gave 84mg (0.20mmol, 88%) of Compound 83 as an oil:
- 20 ¹H NMR (CDCl₃, 300MHz) δ 8.45 (d, *J* = 4.9Hz, 1H), 8.28-8.16 (m, 3H), 7.97 (s, 1H), 7.74-7.68 (m, 1H), 7.64-7.48 (m, 5H), 6.79 (d, *J* = 5.2Hz, 1H), 2.45 (s, 3H).

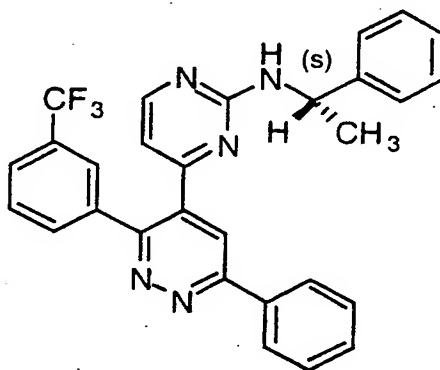


84

4-(2-Methylsulfonylpyrimidin-4-yl)-6-phenyl-3-(3-(trifluoromethyl)phenyl)-pyridazine

Compound **83** (80mg, 0.189mmol), sodium tungstate (7mg, 0.02 mmol), 30% hydrogen peroxide (86 μ L, 0.77mmol), methanol (1mL) and ethyl acetate (5mL) were combined under argon and heated at a gentle reflux for 24h. The mixture was cooled and the solvent was removed *in vacuo* to afford Compound **84**.

¹H NMR (CDCl₃, 300MHz) δ 8.85 (d, J = 5.2Hz, 1H), 8.30 (s, 1H), 8.26-8.18 (m, 2H), 7.96 (s, 1H), 7.78-7.73 (m, 1H), 7.64-7.50 (m, 5H), 7.33 (d, J = 5.2Hz, 1H), 3.30 (s, 3H).



85

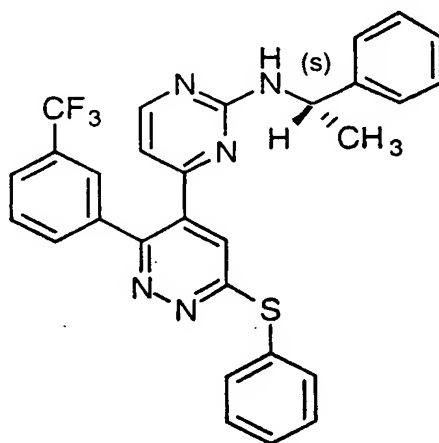
(s)-6-Phenyl-4-[2-(1-phenylethylamino)pyrimidin-4-yl]-3-(3-(trifluoromethyl)phenyl)pyridazine (85)

Compound **84** (0.189mmol) and s-(-)- α -methylbenzylamine (0.500mL) were combined under Ar and heated at 100°C for 1h. The mixture was cooled and

purified by flash column chromatography (hexane:ethyl acetate 70:30) to give an oil which was crystallized using hexane:ethyl acetate ether to give 42mg (0.085mmol, 45%) of Compound 85.

- 5 ¹H NMR (CDCl₃, 300MHz) δ 8.22-8.10 (m, 3H), 7.95 (s, br, 2H), 7.67 (d, *J* = 7.9Hz, 2H), 7.61-7.45 (m, 4H), 7.40-7.25 (m, 5H), 6.29 (d, *J* = 4.9Hz, 1H), 5.57 (d, *J* = 7.3Hz, 1H), 5.10-5.00 (s, br, 1H), 1.58-1.50 (m, 3H).

EXAMPLE 7

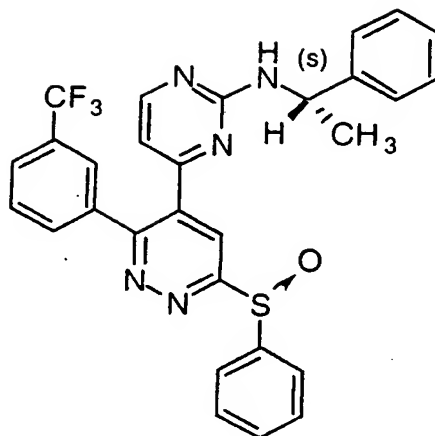


86

(s)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-3-(phenylsulfanyl)-6-(3-trifluoromethylphenyl)pyridazine (86)

- Compound 10 (100 mg, 0.220mmol), thiophenol (~200μL), and diisopropylethylamine (~100mL) were combined under Ar and heated at 120°C for 3h. The mixture was purified by flash column chromatography (hexane:ethyl acetate 70:30) to afford a foam. Crystallization from hexane:ethyl acetate afforded 59mg (0.112mmol, 51%) of Compound 86.

- 20 ¹H NMR (DMSO-d₆, 300MHz) δ 8.30-8.20 (s, br, 1H), 7.90-7.68 (m, 4H), 7.68-7.52 (m, 5H), 7.48 (s, 1H), 7.30-7.10 (m, 5H), 6.56-6.44 (s, br, 1H), 5.20-4.80 (s, br, 1H), 4.40 (s, br, 1H), 1.40-1.00 (m, 3H).



87

(s)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-3-(phenylsulfinyl)-6-(3-trifluoromethylphenyl)pyridazine (87)

5 Compound **86** (70mg, 0.132mmol), sodium periodate (28mg, 0.132 mmol), methanol (2mL) and water (250 μ L) were combined at 0°C under Argon. After 0.5h, the mixture was warmed to room temperature and stirred for 24h. An additional portion of sodium periodate (28mg, 0.132mmol) was added and heated to 60°C for 48h. Ethyl acetate and water were added. The layers were separated and the
10 organic portion was dried with anhydrous sodium sulfate. The solvent was removed *in vacuo* and the remaining residue was purified by flash column chromatography (hexane:ethyl acetate 50:50) to give 20mg (0.036mmol, 28%) of Compound **87** as a foam.

15 ¹H NMR (CD₃OD, 300MHz) δ 8.38-8.30 (m, 1H), 8.22-8.16 (m, 1H), 7.94-7.86 (m, 2H), 7.80-7.70 (m, 2H), 7.66-7.50 (m, 5H), 7.32-7.18 (m, 5H), 6.52-6.44 (s, br, 1H), 1.44-1.36 (m, 3H).

20 The ability of compounds of the present invention to inhibit the synthesis or the activity of cytokines can be demonstrated using the following *in vitro* assays.

BIOLOGICAL ASSAYS

25 Lipopolysaccharide mediated production of cytokines

Human peripheral blood mononuclear cells (PBMC) are isolated from fresh human blood according to the procedure of Chin and Kostura, *J. Immunol.* **151**, 5574-5585 (1993). Whole blood is collected by sterile venipuncture into 60 mL syringes coated with 1.0 mL of sodium- heparin (Upjohn, 1000 U/mL) and diluted 1:1 in Hanks Balanced Salt Solution (Gibco). The erythrocytes are separated from the PBMC's by centrifugation on a Ficoll-Hypaque lymphocyte separation media. The PBMC's are washed three times in Hanks Balanced Salt Solution and then resuspended to a final concentration of 2×10^6 cell/mL in RPMI containing 10% fresh autologous human serum, penicillin streptomycin (10 U/mL) and 0.05% DMSO. Lipopolysaccharide (Salmonella type Re545; Sigma Chemicals) is added to the cells to a final concentration of 100 ng/mL. An aliquot (0.1 mL) of the cells is quickly dispensed into each well of a 96 well plate containing 0.1 mL of the test compound, at the appropriate dilution, and are incubated for 24 hours at 37°C in 5% CO₂. At the end of the culture period, cell culture supernatants are assayed for IL-1 β , TNF- α , IL-6 and PGE2 production using specific ELISA.

IL-1 mediated cytokine production

Human peripheral blood mononuclear cells are isolated from fresh human blood according to the procedure of Chin and Kostura, *J. Immunol.* **151**, 5574-5585 (1993). Whole blood is collected by sterile venipuncture into 60 mL syringes coated with 1.0 mL of sodium- heparin (Upjohn, 1000 U/mL) and diluted 1:1 in Hanks Balanced Salt Solution (Gibco). The erythrocytes are separated from the PBMC's by centrifugation on a Ficoll-Hypaque lymphocyte separation media. The PBMC's are washed three times in Hanks Balanced Salt Solution and then resuspended to a final concentration of 2×10^6 cell/mL in RPMI containing 10% fresh autologous human serum, penicillin streptomycin (10 U/mL) and 0.05% DMSO. Endotoxin free recombinant human IL-1b is then added to a final concentration of 50 pMolar. An aliquot (0.1 mL) of the cells is quickly dispensed into each well of a 96 well plate containing 0.1 mL of the compound at the appropriate dilution and incubated for 24 hours at 37°C in 5% CO₂. At the end of the culture period, cell culture supernatants are assayed for TNF- α , IL-6 and PGE2 synthesis using specific ELISA.

Determination of IL-1 β , TNF- α , IL-6 and Prostanoid
Production from LPS or IL-1 Stimulated PBMC's

IL-1 β ELISA

5 Human IL-1 β can be detected in cell-culture supernatants or whole
blood with the following specific trapping ELISA. 96 well plastic plates (Immulon
4; Dynatech) are coated for 12 hours at 4°C with 1 mg/mL protein-A affinity chroma-
10 tography purified mouse anti-human IL-1 β monoclonal antibody (purchased as
an ascites preparation from LAO Enterprise, Gaithersburg Maryland.) diluted in
Dulbecco's phosphate-buffered saline (-MgCl₂, -CaCl₂). The plates are washed with
PBS-Tween (Kirkegaard and Perry) then blocked with 1% BSA diluent and blocking
solution (Kirkegaard and Perry) for 60 minutes at room temperature followed by
washing with PBS Tween. IL-1 β standards are prepared from purified recombinant
15 IL-1 β produced from *E. coli*. The highest concentration begins at 10 ng/mL followed
by 11 two-fold serial dilutions. For detection of IL-1 β from cell culture supernatants
or blood plasma, 10 - 25 mL of supernatant is added to each test well with 75-90 mL
of PBS Tween. Samples are incubated at room temperature for 2 hours then washed 6
times with PBS Tween on an automated plate washer (Dennly). Rabbit anti-human
20 IL-1 β polyclonal antisera diluted 1:500 in PBS-Tween is added to the plate and
incubated for 1 hour at room temperature followed by six washes with PBS-Tween.
Detection of bound rabbit anti-IL-1 β IgG is accomplished with Fab' fragments of
Goat anti-rabbit IgG-horseradish peroxidase conjugate (Accurate Scientific) diluted
1:10,000 in PBS-Tween. Peroxidase activity was determined using TMB peroxidase
25 substrate kit (Kirkegaard and Perry) with quantitation of color intensity on a 96-well
plate Molecular Devices spectrophotometer set to determine absorbance at 450 nM.
Samples are evaluated using a standard curve of absorbance versus concentration.
Four-parameter logistics analysis generally is used to fit data and obtain
concentrations of unknown compounds.

30 TNF- α ELISA

Immulon 4 (Dynatech) 96-well plastic plates are coated with a 0.5
mg/mL solution of mouse anti-human TNF- α monoclonal antibody. The secondary
antibody is a 1:2500 dilution of a rabbit anti-human TNF- α polyclonal serum
purchased from Genzyme. All other operations are identical to those described above

for IL-1 β . The standards are prepared in PBS-Tween + 10% FBS or HS. Eleven two-fold dilutions are made beginning at 20 ng/mL TNF- α .

IL-6 ELISA

5 Levels of secreted human IL-6 are also determined by specific trapping ELISA as described previously in Chin and Kostura, *J. Immunol.* **151**, 5574-5585 (1993). (Dynatech) ELISA plates are coated with mouse anti-human IL-6 monoclonal antibody diluted to 0.5 mg/mL in PBS. The secondary antibody, a rabbit anti-human IL-6 polyclonal antiserum, is diluted 1:5000 with PBS-Tween. All other operations
10 are identical to those described above for IL-1 β . The standards are prepared in PBS-Tween + 10% FBS or HS. Eleven two-fold dilutions are made beginning at 50 ng/mL IL-6.

PGE₂ production

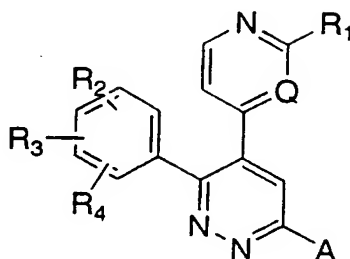
15 Prostaglandin E2 is detected in cell culture supernatants from LPS or IL-1 stimulated PBMC's using a commercially available enzyme immunoassay. The assay purchased from the Cayman Chemical (Catalogue No. 514010) and is run exactly according to the manufacturers instructions.

20 Interleukin-8 (IL-8)

 The present compounds can also be assayed for IL-8 inhibitory activity as discussed below. Primary human umbilical cord endothelial cells (HUVEC) (Cell Systems, Kirkland, Wa) are maintained in culture medium supplemented with 15% fetal bovine serum and 1% CS-HBGF consisting of aFGF
25 and heparin. The cells are then diluted 20-fold before being plated (250 μ l) into gelatin coated 96-well plates. Prior to use, culture medium is replaced with fresh medium (200 μ l). Buffer or test compound (25 μ l, at appropriate concentrations) is then added to each well in quadruplicate wells and the plates incubated for 6h in a humidified incubator at 37°C in an atmosphere of 5% CO₂. At the end of the
30 incubation period, supernatant is removed and assayed for IL-8 concentration using an IL-8 ELISA kit obtained from R&D Systems (Minneapolis, MN). All data is presented as mean value (ng/mL) of multiple samples based on the standard curve. IC50 values where appropriate are generated by non-linear regression analysis.

WHAT IS CLAIMED IS:

1. A compound of the formula



(I)

5 wherein

A is halogen, phenyl, S(O)_m phenyl, or NR₅R₆;

10 R₁ is hydrogen, NH(C₁-C₆ alkyl)aryl, NH(C₁-C₆ alkyl) or NH(C₃-C₆ cycloalkyl), said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF₃, NH₂, and NO₂;

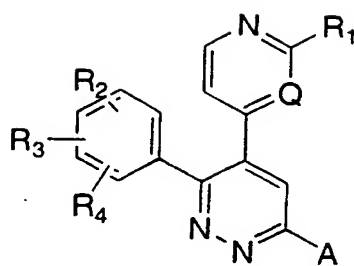
15 R₂, R₃ and R₄ independently represent a member selected from the group consisting of hydrogen, halogen, hydroxy, CF₃, NH₂, NO₂, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₈ cycloalkyl or phenyl;

20 R₅ and R₆ are independently hydrogen, C₁-C₆ alkyl, (C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), (C₁-C₆ alkyl) cycloalkyl, (C₁-C₆ alkyl) NR₇R₈, C₁-C₆ alkylphenyl, said phenyl group optionally substituted with 1 to 3 groups selected from (C₁-C₆ alkyl) or (C₁-C₆ alkoxy); (C₁-C₆ alkyl)-NHCOO-(C₁-C₆ alkyl), (C₁-C₆ alkyl)C≡C, (C₁-C₆ alkyl)indole, (C₁-C₆ alkyl) pyridinyl, a pyrrolidinyl or piperidyl group, said groups optionally substituted with C₁-C₆ alkyl or benzyl; or

25 R₅ and R₆ are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring

- 5 containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-2 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1-C_6 alkyl, OH, $O(C_1-C_6 \text{ alkyl})$, $COO(C_1-C_6 \text{ alkyl})$, C_1-C_6 alkyl benzodioxole, $CONR_7R_8$, phenyl, said phenyl group optionally substituted with halogen, C_1-C_6 alkyl, C_1-C_6 alkoxy; $CH(aryl)_2$ said aryl optionally substituted with 1-3 groups selected from C_1-C_6 alkyl, OH or halogen; NR_7R_8 or a piperidino or a pyrrolidino group;
- 10 R_7 and R_8 are independently hydrogen, C_1-C_6 alkyl, $(C_1-C_6 \text{ alkyl})-O-(C_1-C_6 \text{ alkyl})$, C_1-C_6 alkylaryl, $(C_1-C_6 \text{ alkyl})-NHCOO-(C_1-C_6 \text{ alkyl})$, $COO-(C_1-C_6 \text{ alkyl})$, a pyrrolidinyl or piperidyl group, said groups optionally substituted with C_1-C_6 alkyl or C_1-C_6 alkylaryl; or
- 15 R_7 and R_8 are taken together with the nitrogen atom to form an optionally substituted 4 to 10 membered mono, bicyclic or azabicyclic heterocyclic ring containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atoms, said ring optionally substituted by 1-3 groups selected from C_1-C_4 alkyl, OH, $O(C_1-C_6 \text{ alkyl})$,
- 20 Q is CH or N;
- m is 0, 1 or 2;
- 25 or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

2. The compound in accordance with claim 1 of the formula



(I)

wherein

A is NR_5R_6 ;

5 R_1 is $\text{NH}(\text{C}_1\text{-C}_6 \text{ alkyl})\text{aryl}$, said aryl group being optionally substituted by 1-3 groups selected from halogen, hydroxy, CF_3 , NH_2 , and NO_2 ;

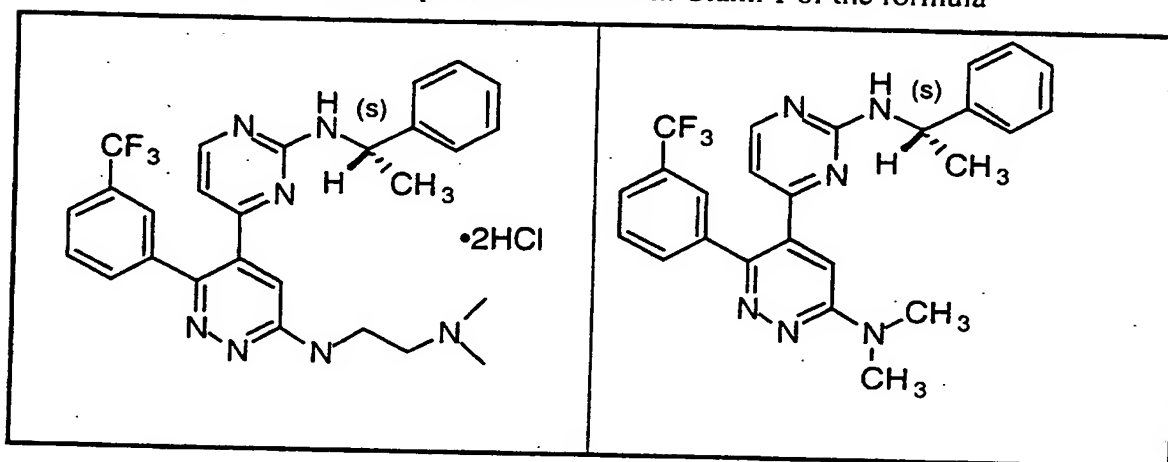
10 R_2 is CF_3 ;

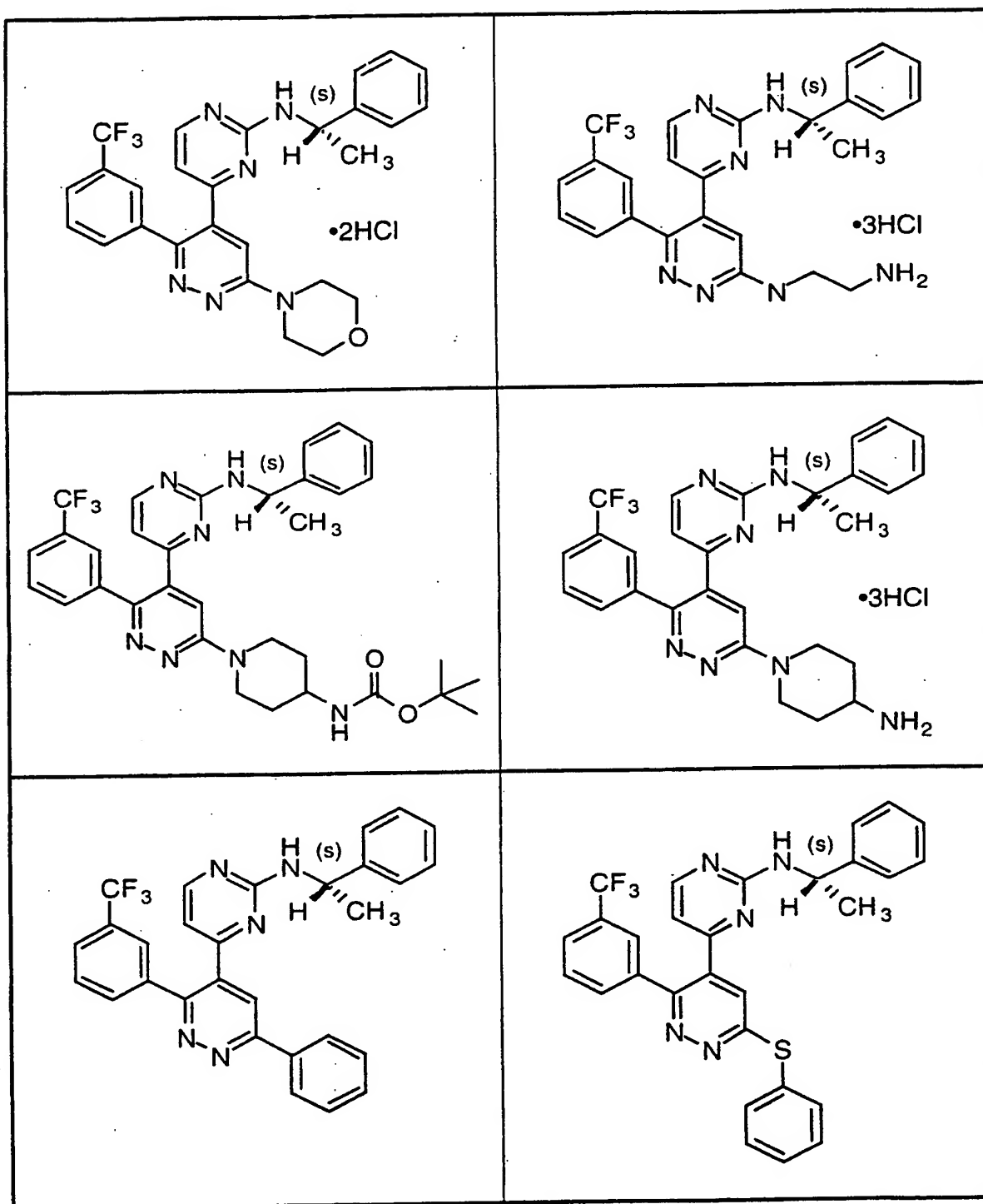
Q is N;

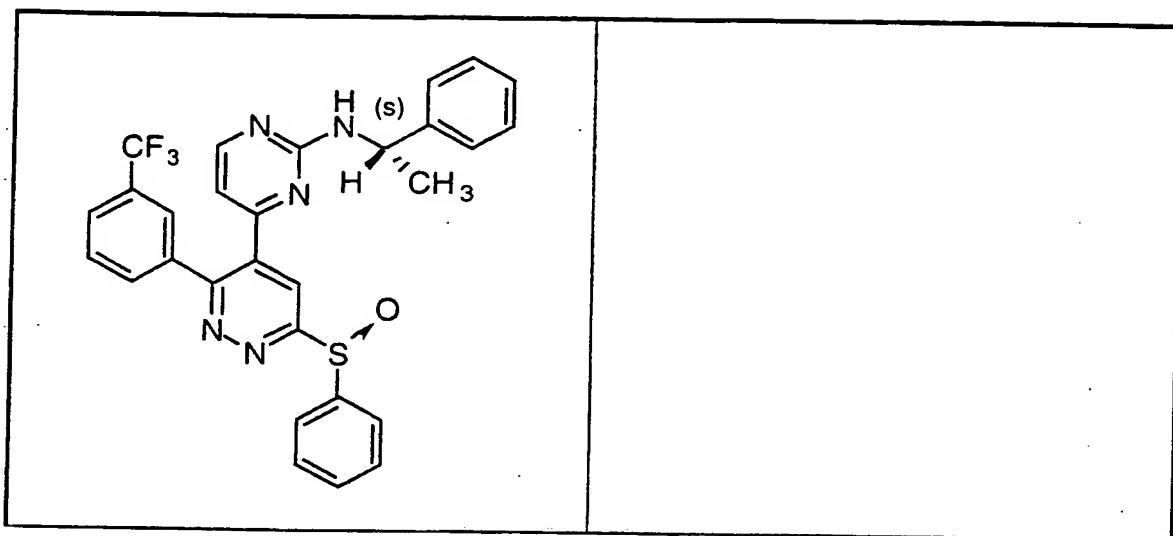
or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

15

3. A compound as defined in Claim 1 of the formula



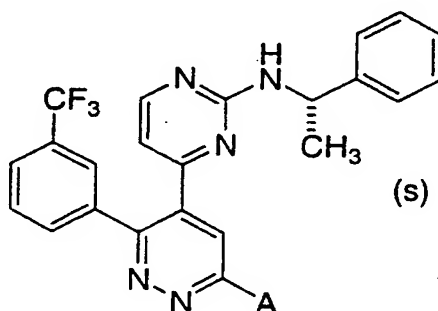




or a pharmaceutically acceptable addition salt and/or hydrate thereof, or where applicable, a geometric or optical isomer or racemic mixture thereof.

5

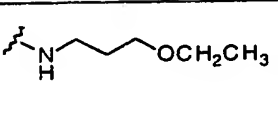
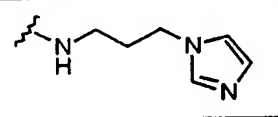
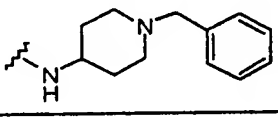
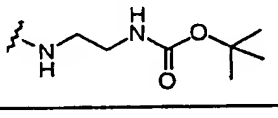
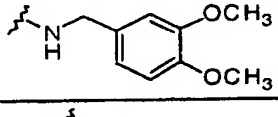
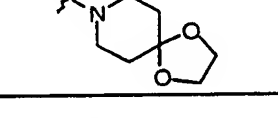
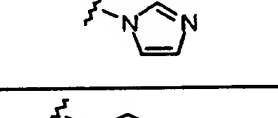
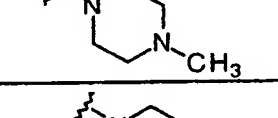
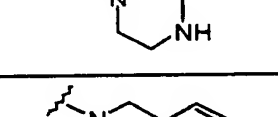
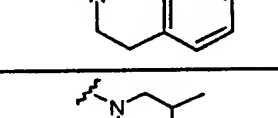
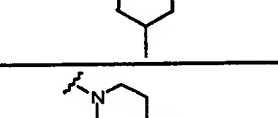
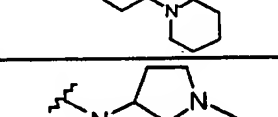
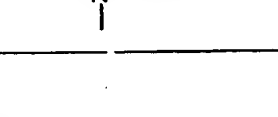
4. A compound as defined in Claim 1 of the formula

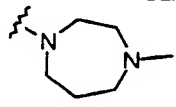
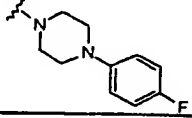
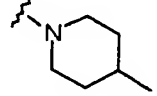
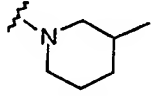
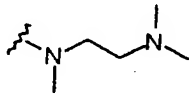
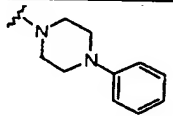
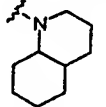
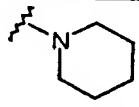
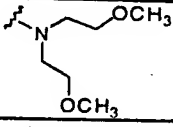
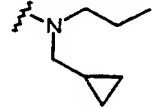
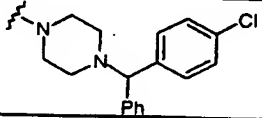
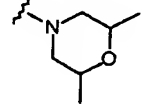
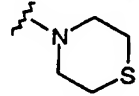


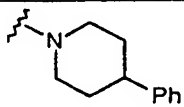
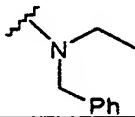
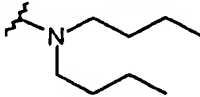
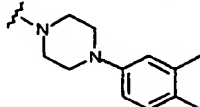
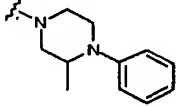
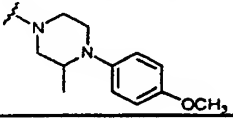
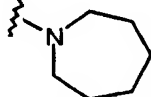
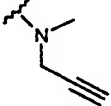
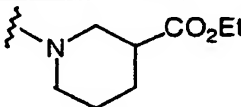
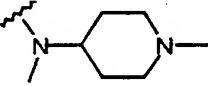
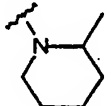
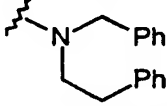
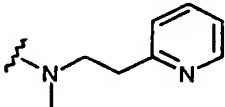
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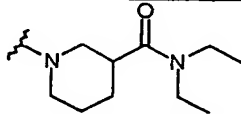
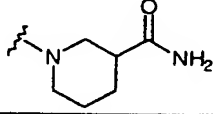
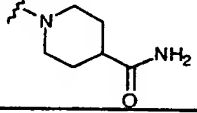
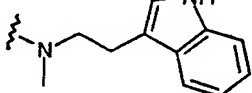
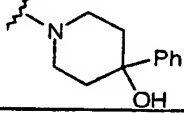
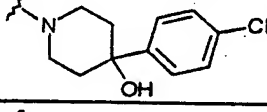
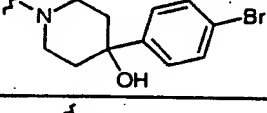
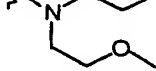
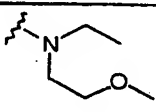
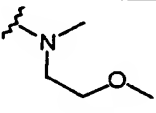
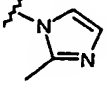
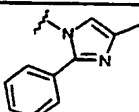
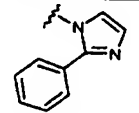
10 wherein A is as indicated in the following table:

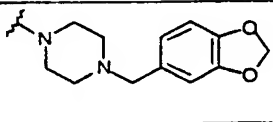
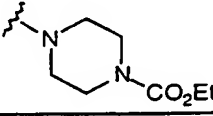
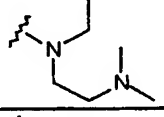
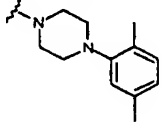
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5. A pharmaceutical composition which is comprised of a compound in accordance with claim 1 in combination with a pharmaceutically acceptable carrier.

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6. A pharmaceutical composition which is produced by combining a compound in accordance with claim 1 and a pharmaceutically acceptable carrier.

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7. A method of treating a cytokine mediated disease in a mammal, comprising administering to a mammalian patient in need of such treatment an amount of a compound as described in claim 1 in an amount which is effective to treat said cytokine mediated disease.

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8. A method of treating inflammation in a mammalian patient in need of such treatment, comprising administering to said patient an anti-inflammatory effective amount of a compound as described in claim 1.

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9. A method in accordance with claim 6 wherein the cytokine mediated disease is rheumatoid arthritis, osteoarthritis, endotoxemia, toxic shock syndrome, inflammatory bowel disease, tuberculosis, atherosclerosis, muscle

degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, rheumatoid arthritis, gout, traumatic arthritis, rubella arthritis or acute synovitis.

10. A method in accordance with claim 7 wherein the cytokine
5 mediated disease is rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcosis, bone resorption diseases, reperfusion injury, graft v. host rejection, allograft rejection, fever, myalgia due to
10 infection, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis or pyresis.

11. A method of treating osteoporosis in a mammalian patient in
15 need of such treatment, comprising administering to said patient an amount of a compound as described in claim 1 which is effective to treat osteoporosis.

12. A method of treating bone resorption in a mammalian patient in
20 need of such treatment, comprising administering to said patient an amount of a compound as described in claim 1 which is effective to treat bone resorption.

13. A method of treating Crohn's disease in a mammalian patient in
25 need of such treatment comprising administering to said patient an amount of a compound as described in claim 1 which is effective to treat Crohn's disease.

14. A process for making a pharmaceutical composition
comprising combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/33097

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/60,122,230,238; 540/601; 514/217.06, 227.8, 236.5, 252.02, 252.03.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE STRUCTURE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|---|------------------------|
| X --- Y | WO 99/32448 A1 (AMGEN INC.) 01 July 1999, see pages 1-39 especially examples in Table on pages 37-39 and example 6A on page 97. | 1,5-14 ----- 2,4 |

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier document published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "G" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

09 FEBRUARY 2001

Date of mailing of the international search report

10 APR 2001

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33097

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07D 401/14, 403/04, 403/14, 413/14, 417/14, 491/118; A61K 31/501, 31/506, 31/5377, 31/541, 31/55.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

544/60, 122, 230, 238; 540/601; 514/217.06, 227.8, 236.5, 252.02, 252.03.